# EFFECTS OF CHROMIUM COMPOUNDS ON HUMAN HEALTH

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This document was prepared under a contract from the Occupational Health and Safety Division of the Ontario Ministry of Labour. 400 University Ave., Toronto, Ontario, Canada M7A 1T7.

ISBN 0-7729-0193-7

88-02119/

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by

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December 1, 1984

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# 1. EXECUTIVE SUMMARY

1.1 Occupational exposures to chromium compounds occur in the following major industries: monochromate and dichromate production, chromate pigment production, chromium electroplating, ferrochromium production, stainless steel welding, spray painting, leather tanning, and cement production and use. Except tanners, the workers in these various industries are exposed to significant amounts of hexavalent chromium (chromates). Mixed exposure to hexavalent and trivalent chromium compounds occur for tanning, chromate and ferrochrome production, and welding; concomitant exposure to chromium metal or chromium alloys exists for welders, ferrochrome and other alloy workers.

1.2 Strong evidence for respiratory cancer has been established for chromate production and chromate pigment production workers, with the data for chromeplaters being somewhat less certain; that for ferrochromium workers, welders, and spray painters may be considered equivocal. Cancers at other sites, specifically cancers of the gastrointestinal tract, nasal and laryngeal cancers, have been noted to be slightly increased, but this finding has not been consistently reported.

1.3 Bioassays and related *in vitro* experiments indicate that all hexavalent chromates (both soluble and insoluble) can induce mutations and inflict chromosomal damage. Positive animal carcinogenicity data are only convincing for calcium chromate (intermediate solubility), lead chromate (sparingly soluble), and perhaps for a number of other sparingly soluble compounds [zinc, strontium and chromium(III) chromates]. For trivalent chromium, the animal studies show no consistent carcinogenic potential; and only when large concentrations are employed, or when a special ligand environment is generated around Cr(III), can mutagenicity be demonstrated.

1.4 Chromium is the second most common skin allergen in the general population, after nickel. In men it is the most frequent sensitizer. The prevalence of chromium dermatitis is the highest in construction workers using cement. Both Cr(III) and Cr(VI) compounds are allergenic, although the former have inferior eliciting capacities in epicutaneous applications. Respiratory sensitivity to chromium compounds is known, but it occurs infrequently.

1.5 There is a high prevalence of ulcerations of the skin and nasal septum among individuals exposed to chromium(VI) compounds. Ambient levels of water-soluble chromate below 0.015 mg Cr $m^{-3}$ are implicated.

1.6 Other than acute pneumonitis associated with heavy exposure to chromic acid, there is little evidence for non-malignant, chronic pulmonary disease from long-term exposure to chromium compounds.

1.7 The prevalence among workers exposed to chromium compounds of gastrointestinal, hepatic and renal impairments (even in mild form) is not documented, and is uncertain.

1.8 There is no human evidence that chromium compounds are associated with birth defects and reproductive deficits in man. Animal experiments have demonstrated that chromium(VI) and chromium(III) compounds can be spermicidal, embryocidal and teratogenic. Placental transport of chromium has been demonstrated in rats for both oxidation states.

1.9 Water-soluble ambient chromium(VI) concentration is a good index to the short-term release of chromium into the urine. The daily incremental increase in urine chromium (post-shift value minus pre-shift value) appears to be a better indicator of current exposure to water-soluble Cr(VI) than the end-of-shift value, because of the large contribution of long-term excretion. In view of animal exposure studies, which showed rapid absorption of sparingly soluble metal chromates, the measurement of total ambient Cr(VI) seems inherently of greater biological significance than an evaluation of water-soluble Cr(VI). Supplementary to the total Cr(VI) level, the total ambient chromium level (all forms) appears to be a suitable index to the contaminant pool building up in the body, such as in welders.

1.10 Dose-response data available from epidemiologic and animal studies of chromium carcinogenicity are of limited value in quantitative risk assessment. Further, there is little evidence that the prevalence of chromium dermatitis correlates with dose. And finally, even very low atmospheric levels of water-soluble chromium(VI) compounds (e.g., $\leq 0.015$ mg Cr $m^{-3}$) are associated with ulcerations of the skin and nasal septum.

# 2. SUBJECT-SPECIFIC SUMMARIES

## 2.1 Chromium Compounds Encountered in Industry

Industrial exposure to a number of specific compounds is documented. Hexavalent compounds include: sodium chromate ($Na_2CrO_4$), sodium and potassium dichromates ($Na_2Cr_2O_7$ and $K_2Cr_2O_7$), and calcium chromate ($CaCrO_4$) in chromate production; chromium(VI) oxide or 'chromic acid' ($CrO_3$) in chromeplating; the pigment chromates of zinc (e.g., $ZnCrO_4$, $Zn_2CrO_4(OH)_2$ and zinc yellow) and of lead (e.g., $PbCrO_4$; $PbO \cdot PbCrO_4$) and water-soluble chromates of welding fumes and ferrochrome exposures. The following trivalent chromium compounds have been identified: chromite ore ($FeO \cdot Cr_2O_3$) in chromate production and the ferrochrome industry; $Cr_2O_3$ in chrome pigment production; and chromium(III) sulphate ($Cr_2(SO_4)_3$) and basic chromium(III) sulphates (e.g., $Cr(OH)SO_4$ and $Cr_2(OH)_4SO_4$) in tanning. In addition, exposures to chromium metal and its alloys take place in the production and use of these products. Industrial applications, and thus exposures to, of chromyl compounds (e.g., $CrO_2Cl_2$) and chemicals containing chromium in less stable oxidation states (e.g., organochromium compounds) appear to be minimal.

## 2.2 Chemical and Biological Aspects of Chromium

### 2.2.1 Oxidation States

Chromium exists in a number of oxidation states, all of which are not of equal stability. Aqueous chromium chemistry and chromium biochemistry are mainly concerned with hexavalent and trivalent chromium.

### 2.2.2 The Chromate Ion (Hexavalent)

The chromate ion, $CrO_4^{2-}$, is taken up readily by cells and tissues. It is reduced extracellularly and intracellularly to the Cr(III) ion, either enzymatically or directly by reductants. The biological half-life of chromate is estimated to be minutes to hours. The dichromate ion, $Cr_2O_7^{2-}$, dissociates completely to the chromate ion at physiologic pH and concentrations below $10^{-2}$ M.

### 2.2.3 The Chromium(III) Ion

The Cr(III) ion tends to be excluded from cells, unless placed in special ligand environments. It forms highly stable metal-ion complexes with proteins, polynucleotides (eg. RNA and DNA) and low-molecular-weight biomolecules, often inhibiting biological function in consequence.

### 2.2.4 Water Solubility of Metal Chromates

Metal chromates may be divided into three arbitrary categories according to water solubility at ambient temperature. The first group may be designated as sparingly soluble to insoluble and includes chromates of zinc, lead, barium and strontium ( $\leq 1$ g $l^{-1}$ or $\leq 10^{-2}$ moles $l^{-1}$). Group two, which displays intermediate (moderate) solubility, consists of potassium dichromate and calcium chromate (50 - 150 g $l^{-1}$ or 0.2 - 1 moles $l^{-1}$). And thirdly, a highly soluble group may be identified which includes potassium and sodium chromates and sodium dichromate (600 - 2500 g $l^{-1}$ or 3 - 10 moles $l^{-1}$). Because of the reducing power and chromium(III)-chelating ability of biological media, water solubility is not a good index to the rate of internal mobilization and pathways of metal chromates.

### 2.2.5 Chromium Absorption in Individuals not Occupationally Exposed

Chromium is poorly absorbed from food ($\leq$ 1%), as are chromium(III) salts when taken orally (~0.5%). Water-soluble hexavalent chromium compounds (chromates) when taken by mouth are absorbed at a higher rate (~10%).

### 2.2.6 Chromium Absorption in Occupationally Exposed Individuals

The main route of exposure is inhalation (including of course inhalation/ingestion interchange), while dermal absorption of dissolved chromate is known. Dose-response relationships between water-soluble ambient chromium(VI) and urinary chromium concentrations have been documented.

### 2.2.7 Chromium Distribution in Man

Chromium, taken as Cr(III) or Cr(VI), is primarily localized in blood, liver, spleen, body soft tissues, and bone. It is transported bound to the iron plasma-protein transferrin.

### 2.2.8 Chromium Excretion in Man

Chromium is excreted in both the urine and faeces. "Uptake" compartments accumulate and release chromium relatively rapidly, with half-times of 1h to 2 days; while "storage" compartment chromium is taken up and decays very slowly (half-times of 4 days and 300 days, respectively).

### 2.2.9 Chromium Analysis

It is technically feasible to determine chromium in body fluids, tissues and air samples. Rigorous control of contamination during collection, handling and analysis of specimens is essential. Creatinine adjustment of urinary chromium concentrations appears to compensate for differences in urine flow rates. Plasma chromium levels are about 15% of the corresponding urinary concentrations.

### 2.2.10 Chromium in Body Fluids and Tissues

Except for hair, much of the chromium concentration data reported prior to 1980 for body fluids and tissues is unreliable because of contamination (extraneous addition) during specimen collection, handling and analysis.

### 2.2.11 Environmental and Biological Monitoring

Water-soluble ambient chromium(VI) concentration is a good index to the short-term release of chromium into the urine. The daily incremental increase in urine chromium (post-shift level minus pre-shift level) appears to be a better indication of current exposure to water-soluble chromium(VI) compounds than the end-of-shift value, because of the large contribution of long-term excretion. In view of animal exposure studies, which showed rapid absorption of sparingly soluble metal chromates, the measurement of total ambient Cr(VI) seems inherently of greater biological significance than an evaluation of water-soluble Cr(VI).

Supplementary to the total Cr(VI) level, the total ambient chromium concentration (all forms) appears to be a suitable index to the contaminant pool building up in the body, such as in welders.

## 2.3 Chromium Carcinogenicity

### 2.3.1 Summary of Epidemiological Evidence

The risk of lung cancer has been consistently shown to be high in the chromate manufacturing industry, where exposure is to a mixture of insoluble (largely trivalent) and both moderately soluble and soluble (largely hexavalent) chromium compounds. The data suggest that even the more recently employed workers, who presumably are exposed to lower levels of chromium than workers employed previously, may have an excess cancer risk with prolonged employment.

In the chromate pigment production industry, where the exposure is largely to sparingly soluble hexavalent compounds, high risk was also demonstrated in several studies. Moreover, one report indicated that the cancer hazard associated with production of chrome pigments may extend to the users of these pigments, such as spray painters.

In the ferrochromium producing industry, where workers are exposed to chromium which predominantly is metallic and trivalent, but nevertheless contains 10-30% soluble hexavalent chromates, the possibility of increased risk for lung cancer has also been raised. However, the relative risks reported were considerably lower than those generally experienced in chromate production. Since there have been conflicting reports, and in view of various methodological problems in the studies, the findings should be regarded as tentative.

Similarly, an increased risk of lung cancer has also been reported for chromium platers exposed primarily to highly soluble chromates. Again, the limited number of studies and limited strength and consistency of the association leave some room to question these findings, but the suggestion of a dose-response relationship in several of these studies is certainly noteworthy.

Various studies have indicated that welders in general are at increased risk of cancer. Since there is as yet little epidemiological data on selected subcohorts of welders with restricted exposures, it has been suggested that supposition of process-dependent "hot spots" of risk must be sought on the basis of indirect (i.e., non-human) experiments. However, in one recent epidemiological study, it has indeed been suggested that increased cancer risk is most strongly associated with stainless steel welding, specifically implicating chromium exposure as a causative factor. It has also been estimated that the chromium-induced risk ratio for respiratory tract cancer among stainless steel welders resulting from cumulative exposures of the order of 3 (mg $m^{-3}$).years may be near 6.

An excess of cancers at sites other than the respiratory system has been reported in chromate production workers, chromium platers, chromate pigment producers, and ferrochromium production workers. Specifically, cancers of the gastrointestinal tract, as well as nasal and laryngeal cancers, have been noted to be slightly increased in various of these investigations. Such findings, however, have not been consistently found in all studies, and no firm conclusions are possible.

### 2.3.2 Bioassays and Animal Studies

Bioassays and related *in vitro* experiments indicate that all hexavalent chromates (both water-soluble and insoluble) can induce mutations and inflict chromosomal damage. In contrast, positive animal carcinogenicity data is only convincing for $CaCrO_4$ (intermediate solubility) and $PbCrO_4$ (sparingly soluble); and perhaps also for the sparingly soluble compounds $ZnCrO_4$, $SrCrO_4$ and $Cr_2(CrO_4)_3$, although only a few experiments have been reported for these. Retention, clearance and absorption in lung of metal chromates would appear to vary with composition, solid surface morphology and reactivity, solubility, and particle size of ambient particulates. The results for soluble chromates are negative. For trivalent chromium, the animal studies show no consistent carcinogenic potential; and only when large concentrations are employed, or when a special ligand environment is generated around Cr(III), can mutagenicity be demonstrated.

### 2.3.3 Classification of Chromium Compounds as "Carcinogenic" and "Non-Carcinogenic"

We conclude that the current integrated evidence does not permit the division of chromium compounds into carcinogenic and non-carcinogenic. It is clear that the sparingly soluble chromates (hexavalent) have recognized carcinogenic potency, while incriminating evidence is mounting for soluble chromates (hexavalent). Although the projected risk associated with exposure to trivalent chromium does not seem to be of the same order of magnitude as hexavalent chromium, it cannot be concluded that Cr(III) compounds are devoid of carcinogenic potential. Since not much is known about exposures to chromium metal, no conclusions are possible.

## 2.4 Chromium Hypersensitivity

### 2.4.1 Clinical Considerations

Chromium is the second most common skin allergen in the general population, after nickel. In men it is the most frequent sensitizer. The prevalence of chromium dermatitis is the highest in construction workers using cement. Other occupational exposures associated with chromium sensitivity are numerous, of which chromium plating, tanning of leather, application of anti-corrosive agents, and printing are representative industrial activities. There is little evidence that the prevalence of chromium dermatitis, correlates with dose. The consensus is that the outlook for persons with chromium dermatitis is bleak. Avoidance strategies, including replacement of chromium-containing constituents and addition of suppressors, show promise and have been implemented in a few instances. Although respiratory sensitivity to chromium compounds is known, it occurs infrequently.

### 2.4.2 Pathogenesis

In terms of pathogenesis, chromium contact sensitivity of the skin is a characteristic cell-mediated phenomenon (delayed hypersensitivity). Hexavalent chromium compounds generally are more potent inducers and elicitors of sensitivity than trivalent compounds; however, the Cr(III) ion very likely figures in the formation of the ultimate and common antigenic determinant. Concentration, solubility, pH and presence/absence of reducing agents are significant influences on the likelihood and intensity of sensitivity associated with contact exposure to chromium compounds. There is no clear evidence that chromium hypersensitivity is strongly genetically determined in humans. Oral ingestion of chromium can lead to skin reactions in sensitive subjects, but the overall significance of non-cutaneous absorption of chromium is uncertain with respect to induction and elicitation of sensitivity and production of tolerance. Sensitivity to light is a potentiating factor in some subjects. The existence of cross-sensitivity to other salts has not been excluded, but many "double allergies" could plausibly derive from the development of separate, independent sensitivity states. Animal studies would suggest that the prospects for desensitizing interventions in humans are poor. It is not known whether chronic exposure to chromium results in desensitization in man. The induction of tolerance may be taking place to an uncertain extent in the community and in workplaces, and has been postulated to be a dose-related phenomenon.

## 2.5 Other Health Effects

### 2.5.1 Ulcerations of the Skin and Nasal Tissues

Chromate dust and chromic acid mist are responsible for the high prevalence of skin and nasal septum ulcerations among occupationally exposed individuals. Even very low atmospheric levels of water-soluble chromium(VI) compounds are associated with such skin and intranasal lesions (e.g. $\leq 0.015$ mg Cr $m^{-3}$).

### 2.5.2 Long-Term Pulmonary Sequelae

Other than acute chemical pneumonitis, which accompanies heavy exposure to chromic acid, little evidence exists to confirm the existence of non-malignant, chronic pulmonary disease from long-term exposure to chromium compounds.

### 2.5.3 Gastrointestinal, Hepatic and Renal Dysfunctions

Although gastrointestinal, hepatic and renal impairments occur on acute chromate poisoning, their prevalence among workers is not well documented and is uncertain (even in mild form).

### 2.5.4 Reproductive Deficits

There is no human evidence that chromium compounds are associated with birth defects, or other reproductive deficits. Animal experiments have demonstrated that chromium(VI) and chromium(III) compounds can be spermicidal, embryocidal and teratogenic. Placental transport of chromium has been demonstrated in rats for both oxidation states.

## 2.6 Comments on Dose-Response Relationships

### 2.6.1 Carcinogenicity

Intramuscular and intrapleural administration of calcium chromate to rodents has shown that low doses were less effective than high doses in malignant tumour induction. Data were insufficient to test for a dose-response relationship. By contrast, similar administration of other metal chromates indicated that the tumour yield increased systematically with dose (expressed in mg of chromium), independent of the metal counter ion ($Cr^{3+}$, $Pb^{2+}$, $Sr^{2+}$ or $Zn^{2+}$) and mode of administration (intramuscular or intrapleural). A threshold value of 2-4 mg Cr was suggested. However, the apparent dose-response relationship must be viewed with caution as the number of data points for each metal chromate and mode of application was few, and must therefore be considered of limited value in quantitative human risk assessment.

Unfortunately, available worker exposure data do not match the period of exposure which has been the focus of health studies. Consequently, it must be concluded that the dose-response data available from epidemiological studies are inadequate to allow a quantitative risk assessment, which of course precludes an evaluation of risk at low levels of exposure. However, the epidemiologic data reviewed do provide some indirect evidence concerning dose-response relationships. Using the duration of employment as an indicator of exposure, authors were frequently able to demonstrate that workers employed longer had a higher risk of respiratory cancer. The associated average latency period, estimated from the interval between initial employment to the date of cancer diagnosis was estimated to be 18 ± 5 y.

### 2.6.2 Hypersensitivity

As for other contact dermatitides, little is known whether sensitization (induction) of chromium contact dermatitis by either chromium(III) or chromium(VI) compounds is dose dependent. If such a relationship were indeed operative, it might be complicated by desensitization in sensitized individuals and by the development of tolerance in non-sensitized (naive) subjects. By contrast, elicitation in chromium-hypersensitive patients by patch testing is dependent on the concentration of the chromium(III) or chromium(VI) compound applied. The threshold concentrations of hexavalent compounds were lower than those of trivalent chromium salts. Data are not available to construct similar dose-response relationships for occupational and environmental exposures.

There is no evidence in the literature to allow estimation of doses required for induction or elicitation of occupational asthma induced by chromates.

### 2.6.3 Skin and Nasal Tissue Ulcerations

Ambient levels of water-soluble chromium(VI) compounds less than 0.015 mg Cr $m^{-3}$ are implicated in the prevalence of ulcerations of the skin and the nasal septum.

### 2.6.4 Urinary Chromium Concentration as an Index to Exposure

The daily incremental increase in urinary chromium concentration (post-shift level minus pre-shift level) is a good index to current exposures to water-soluble chromium(VI) compounds. The following relationships summarize the observed data: $\Delta$Cr (urine, μg per g of creatinine) = 200 Cr (air, mg $m^{-3}$); or $\Delta$Cr (urine, μg $l^{-1}$) = 300 Cr (air, mg $m^{-3}$).

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# 3. Chemical and Biological Aspects of Chromium

## 3.1 Forms of Chromium Encountered in Industry

The flow chart provided in Fig. 3.1 indicates that workers are exposed to a limited number of chromium compounds. Included in this list, or implied by it, are the following hexavalent compounds: sodium chromate ($Na_2CrO_4$) and sodium dichromate ($Na_2Cr_2O_7 \cdot 2H_2O$); calcium chromate ($CaCrO_4$); the pigment chromates including those of zinc (e.g., $ZnCrO_4$, $Zn_2CrO_4(OH)_2$, zinc yellow ) and lead ($PbCrO_4$, $PbO \cdot PbCrO_4$); and the chromium(VI) oxide ($CrO_3$), which colloquially is called "chromic acid". Exposure to trivalent chromium is in the following forms: chromite ore ($FeO \cdot Cr_2O_3$) and calcium chromite ($CaCr_2O_4$); chromium(III) oxide ($Cr_2O_3$); chromium and basic chromium sulphates, e.g., $Cr_2(SO_4)_3$, $Cr(OH)(SO_4)$, $Cr_2(OH)_4(SO_4)$, etc; and chromium metal and alloys (Gafafer, 1953; Foley, 1978; Morgan, 1978; Stern, 1982).

As may be surmised from the above listing, chromium toxicity and thus risk assessment is dependent on its chemical form. A brief description of the rudiments of the aqueous chemistry of chromium is therefore warranted. The coverage is divided into three components; namely, (1) stable oxidation states; (2) chromium(VI) chemistry; (3) chromium(III) chemistry; and (4) the chemistry of less stable oxidation states.

## 3.2 Chromium Chemistry

### 3.2.1 Stable Oxidation States

Chromium exists in a number of oxidation states, all of which are not of equal stability. This is depicted in Fig. 3.2. As indicated in the legend of this figure, thermodynamic energy considerations show that positive values of the standard electrode potential ($E^o$) indicate that the reduced form is favoured, while negative $E^o$ values denote that the oxidized species is relatively more stable*. Consequently, it is clear from this reduction-potential diagram that Cr(III) is the most stable form of chromium in solution, as considerable energy would be required to convert it to lower or higher oxidation states. While $CrO_4^{2-}$ is relatively stable, its high positive reduction potential denotes that it is strongly oxidizing and is unstable in acid solution in the presence of electron donors such as $Fe^{2+}$, $H_3AsO_3$, $HSO_3^-$, and all organic molecules with oxidizable groups (alkanes, alkenes, alcohols, aldehydes, ketones, carboxylic acids, mercaptans, etc.; Wiberg, 1965; Beattie and Haight, 1972). At physiologic pH values or higher, $CrO_4^{2-}$ is less reactive as indicated by the relatively low $E^{o'}$ values in Table 3.1 (also see Fig. 3.2)**. Presumably it is for this reason that gross cellular destruction does not occur in bioassays. Chromium(V) species are derived from the anion $CrO_4^{3-}$, and are long-lived enough to be observed directly. However, there are relatively few stable compounds containing Cr(V). Chromium(IV) compounds are even less common. The Cr(IV) ion and its compounds are not very stable, and because of short half-lives, defy detection as reaction intermediates between $CrO_4^{2-}$ and Cr(III). It should be emphasized that $CrO_4^{2-}$ and $CrO_4^{3-}$ are anions (like carbonate, $CO_3^{2-}$, and sulphate, $SO_4^{2-}$) and, in fact, can behave as ligands (e.g., like ammonia, $NH_3$; EDTA; $CO_3^{2-}$, etc.) toward metal ions (e.g., Cu(II), Ni(II), Fe(III) or even the Cr(III) ion). Cr(IV) appears to react as a metal ion. This distinction between anionic and cationic forms of chromium is essential to the understanding of their divergent biological effects.

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* Reduction corresponds to a gain of electrons, while oxidation is accompanied by a loss of electrons. In the absence of kinetic limitations, the oxidizing power increases with the numerical (positive) value of $E^o$ (see Table 3.1 and Fig. 3.2 for additional explanation).

** $E^{o'}$ is the formal electrode potential and refers to non-standard conditions; while $E^o$, the standard electrode potential, corresponds to solutions of unit activity ($\geq 1$ mol $l^{-1}$ for most species).

The negative $E^o$ value for the Cr(III)/Cr(II) metal-ion couple (see Fig. 3.2) signifies that Cr(II) is a strong reducing agent (it readily gives up electrons and thus is itself oxidized to Cr(III)). Only under stringent anaerobic conditions is it stable in aqueous solution; even then, it slowly decomposes by reducing water with liberation of hydrogen gas (Cotton and Wilkinson, 1980,p.724).

### 3.2.2 Chromium(VI) Chemistry

#### 3.2.2.1 Chromate Speciation

Chromium(VI) is strongly oxidizing and exists only in "oxo" species such as $CrO_3$ (chromium(VI) oxide, chromium trioxide, colloquially referred to as chromic acid), $CrO_2Cl_2$ (chromyl chloride) and $CrO_4^{2-}$ (chromate ion). $CrO_3$ is highly soluble in water (625.3 g $l^{-1}$ or 6.25 mol $l^{-1}$ at 20° C), and in basic solution forms the yellow chromate ion; dissolution in acidic medium yields a number of pH and concentration-dependent species. As may be deduced from the acid dissociation constant in Eqn (3.1), $H_2CrO_4$ is a strong acid ($pK_{a1}$ = -0.6) and is the main species below pH -0.6 (Cotton and Wilkinson, 1980,p.733).

$$H_2CrO_4 \rightleftharpoons H^+ + HCrO_4^- \qquad K_{a1} = 10^{0.6} \qquad (3.1)$$

$$HCrO_4^- \rightleftharpoons H^+ + CrO_4^{2-} \qquad K_{a2} = 10^{-5.9} \qquad (3.2)$$

In contrast, the monohydrogen chromate ion ($HCrO_4^-$) is a weak acid ($pK_{a2}$ = 5.9; Eqn 3.2) and is the primary species between pH 1 and 6, with $CrO_4^{2-}$ predominating at pH greater than 6. At concentrations of Cr(VI) above $10^{-2}$ M, the $HCrO_4^-$ ion dimerizes to give the dichromate ion (Baes and Mesmer, 1976; Connett and Wetterhahn, 1983; see Eqn 3.3).

$$Cr_2O_7^{2-} + H_2O \rightleftharpoons 2HCrO_4^- \qquad K = 10^{-2.2} \qquad (3.3)$$

In biological systems the chromate concentrations encountered are likely to be considerably below $10^{-2}$ M, and thus the existence of dichromate is not expected to be significant, especially at physiologic pH.

### 3.2.2.2 Water Solubility of Metal Chromates

Since the carcinogenic potency of chromates have been linked to their water solubility, a brief review of the available data is presented. Metal chromates may be divided into three arbitrary categories, as is illustrated in Table 3.2. The first group may be designated as sparingly soluble to insoluble and includes chromates of zinc, lead, barium and strontium. Of intermediate character are calcium chromate and potassium dichromate. It should be emphasized that solutions of significant concentration of these latter two compounds are possible at ambient temperature (e.g., 0.2 - 1.0 mol $l^{-1}$). Consequently, calcium chromate is considerably more soluble than other metal chromates incriminated in chromium carcinogenesis (i.e., of Pb, Sr, and Zn; see Chapter 4). And finally, the third solubility category includes the highly soluble potassium and sodium chromates, as well as sodium dichromate.

Biological media such as serum and cytosol have a high affinity for metal ions (e.g., $Zn^{2+}$, $Pb^{2+}$, $Ba^{2+}$) and contain reducing agents that can react with chromate. For these reasons, the solubility of metal chromates may be expected to be augmented in biological solutions. The enhancement of solubility by complex formation of the released metal ion has been clearly demonstrated for the dissolution of nickel compounds in rat serum and renal cytosol (Kuehn and Sunderman, 1982). Similar studies have not been reported for metal chromates.

### 3.2.2.3 Chromium(VI) Oxide

It is evident from the flow chart in Fig. 3.1 that chromium(VI) oxide ($CrO_3$, "chromic acid") is an important industrial intermediate (also see IARC, 1980). As already mentioned in Section 3.2.2.1, the solid $CrO_3$ (m.p. 197° C) dissolves readily in water yielding chromic acid ($H_2CrO_4$) and its deprotonated forms ($HCrO_4^-$, $CrO_4^{2-}$) depending on pH. It is the acid anhydride of $H_2CrO_4$, and is prepared industrially by the dehydration with sulphuric acid of sodium dichromate (see Fig. 3.1; Brown, 1974). Because of its high solubility, and because the dissolved forms are powerful oxidizing agents (see Section 3.2.2.4), $CrO_3$ is a primary irritant. Contact with combustible material may cause fire (Merck, 1983). It is obvious that special precautions are required in the handling of this material and these have been outlined by NIOSH (NIOSH 1973, 1975).

### 3.2.2.4 Chemical Reactivity of the Chromate Ion

Extensive use has been made of chromic acid as an oxidizing agent in both organic and inorganic chemistry. Comprehensive reviews on these subjects are available (Wiberg, 1965; Espenson, 1970; Beattie and Haight, 1972). A perusal of these reviews suggests that strongly acidic chromate solutions are powerful oxidants. As illustrated in Section 3.2.1, the oxidizing power of chromate is considerably diminished at physiological pH values. Consequently, a legitimate question is whether the versatility demonstrated in acidic solution applies to biological systems. Recent data have indicated that at pH 7.4 chromate is indeed capable of oxidizing both low-molecular-weight reductants (e.g., cysteine, ascorbate, glutathione, lipoic acid) and proteins. These reactions adhere to second order kinetics, and thus are dependent on the concentration of both the chromate and the reductant (Connett and Wetterhahn, 1983). Since the concentrations of the mentioned low-molecular-weight reductants are often in the range $10^{-5}$ to $10^{-3}$ mol $l^{-1}$ in biological fluids, the published rate constants predict reaction half-times in the range of minutes to hours when chromate is present in comparable concentrations. Connett and Wetterhahn (1983) speculate that because of the high intracellular levels of glutathione (0.8 - 8 mmol $l^{-1}$), it may be the most effective reagent for converting chromate to chromium(III). We may conclude, therefore, that chromate is chemically unstable at physiological pH values, but does not decompose as rapidly as expected on the basis of the highly positive standard reduction potential so frequently quoted in health assessment documents ($E^o$ = 1.33 V). Consequently, a biological half-life for the chromate ion of the order of hours is not unrealistic.

Chemically it is difficult to displace the oxygen atoms from the chromate ion, and it is said to be non-labile kinetically. In contrast, the hydroxyl group of the monoprotonated form of chromate ($HCrO_4^-$; see Fig. 3.3) is more readily replaced. In fact, $HCrO_4^-$ forms esters with other species containing -OH or -SH groups. The formation of the dichromate ion is a prime example. It can be seen from Eqn (3.3) that this involves the elimination of water from the two units being linked. Adducts are known to form with oxyacids (e.g., $HSO_4^-$, $HSO_3^-$, $HS_2O_3^-$, $H_2PO_4^-$), alcohols (R-OH), thiols (R-SH), carboxylic acids (R-COOH), and other anions (e.g., $Cl^-$, $SCN^-$) (Wiberg, 1965; Beattie and Haight, 1972; Connett and Wetterhahn, 1983). Ester formation is favoured in acid solution but is less important at physiological pH values, presumably because of the low concentration of the monoprotonated form of chromate. Nevertheless, recent kinetic studies suggest that the rate-determining step in the oxidation of thiols by chromate at physiological pH is the rate of thioester formation. Consequently, chromate esters may occur as reaction intermediates, and in this manner could be of toxicological consequence.

### 3.2.2.5 Chromyl Chloride (Dichlorodioxochromium(VI))

Chromyl chloride, $CrO_2Cl_2$, is used extensively in organic synthesis (Wiberg, 1965). It is prepared by the action of hydrogen chloride on chromic(VI) oxide or by warming dichromate with an alkali metal chloride in concentrated sulphuric acid (Cotton and Wilkinson, 1980,p.734). Because of the latter reactions, the use of chromic-sulphuric acid cleaning solution may yield chromyl chloride (King,1978). This compound is an unusual chromium(VI) derivative in that it is a liquid at room temperature (b.p. 116° C) and is soluble in organic solvents (Hartford and Darrin, 1958). It undergoes hydrolysis on contact with water, yielding $H_2CrO_4$ (see Eqn (3.4)), or its dissociation products $HCrO_4^-$ and $CrO_4^{2-}$.

$$CrO_2Cl_2(l) + 2H_2O(l) \rightleftharpoons H_2CrO_4(aq) + 2HCl(g) \qquad (3.4)$$

This reaction is exothermic and explains the fuming of $CrO_2Cl_2$ in moist air. Furthermore, as an oxidant it can react explosively with combustible substances. Hydrolysis and oxidation likely are the cause of the burns and blisters on contact with skin (or other tissues for that matter).

Chromyl compounds of other halides (especially fluoride), and of other anions (e.g., nitrate, cyanate, acetate) are known (Hartford and Darrin, 1958; Wasson, 1976). These chromyl compounds, like the chloride derivative, are volatile materials, although there are wide differences in their boiling points and volatilities. Chromyl chloride serves as a prototype with regards to the chemical activity of these compounds. Thus they are important synthetically, and the reactions just described also apply. However, it is difficult to assess the extent of their employment in industry. In any event, extreme care must be exercised when working with chromyl compounds (e.g., containment in a high performance fumehood) because of their high volatility and chemical reactivity.

### 3.2.3 Chromium(III) Chemistry

#### 3.2.3.1 Kinetics of Cr(III)-Complex Formation

As described in Section 3.2.2, the chemistry of chromium(VI) is essentially that of the chromate anion ($CrO_4^{2-}$). Other than in acidic solutions, it is difficult to displace the oxygen atoms from this anion. Consequently, the immediate environment around chromium(VI) (referred to as the coordination polyhedron) is fixed; namely, is occupied by oxide or hydroxide groups. The affinity of chromium(III) for the oxide and hydroxide ions is considerably lower, and numerous complexes with other ligands are possible. Complexes are known with ligands for which the point of attachment is the oxygen atom (e.g., water, hydroxyl ion, carboxylic acids, phenols, phosphates), or the nitrogen atom (e.g., ammonia, amines, pyridines, purines, pyrimidines, imidazoles), or the sulphur atom (e.g., sulphydryl groups). It is largely because of kinetic inertness that so many complex species of chromium(III) can be isolated or are stable in solution. In fact, chromium(III) species are considered non-labile, and ligand displacement is slow (hours to days at room temperature) compared to most metal ions ($10^{-9}$ s to $10^{-3}$ s at room temperature; Cotton and Wilkinson, 1980, pp. 727 and 1188).

From the above deliberations, one should not conclude that reactions of the hydrated Cr(III) ion, $Cr(OH_2)_6^{3+}$, or of other complex species of Cr(III), do not occur. Under selected conditions (e.g., in the presence of a catalyst, by ligand-assisted labilization, or at elevated temperatures) even substitution-inert complexes may be induced to exchange their ligands somewhat more rapidly, and this has been employed synthetically for Cr(III) (Garner and House, 1970; De Pamphilis and Cleland, 1973; also see Sulfab _et al_., 1976). At physiological temperature (37° C), chromium(III) reactions are faster by a factor of 4 - 5 than at ambient temperatures (20 - 25° C). Because of this, and because suitable catalysts are available (e.g., oxidizing/reducing agents), reactions of chromium(III) are plausible in mammalian systems. As explored in Section 3.3, it may be surmised that the non-lability of chromium(III) complexes is of special biological significance.

### 3.2.3.2 Types of Chromium(III) Complexes

Like other trivalent metal ions (e.g., Fe(III), Al(III)), the hydrated Cr(III) ion, $Cr(OH_2)_6^{3+}$, has a tendency to hydrolyze, which is often accompanied by polymerization. Hydrolysis involves the conversion of a bound water molecule to the hydroxide ion and results in the release of a proton. Equilibrium measurements have identified, among others, the existence of the following species: $Cr(OH)^{2+}$, $Cr(OH)_2^{+}$, $Cr(OH)_3(s)$, $Cr_2(OH)_2^{4+}$,

$Cr_3(OH)_4^{5+}$, $Cr_4(OH)_6^{6+}$ (Smith and Martell, 1976; Baes and Mesmer, 1976; Connett and Wetterhahn, 1983). In the polymeric species, the chromium ions are linked by hydroxyl bridges. At pH 4, about one half of the hydrated Cr(III) ions are present as the monohydroxo complex, $Cr(OH)^{2+}$. To prevent this hydrolysis process, Cr(III)-solutions must be stored under acidic conditions ($pH \leq 3$).

Chromium(III), like other first-row transition metal ions, may be designated as a "borderline" ion which possesses considerable class A character. The "borderline" classification denotes that this ion has the ability to combine with O-, N- and S- containing ligands, in which the designated donor atoms are the point of attachment (Nieboer and Richardson, 1980). This catholic affinity is the basis for the multiplicity of possible complexes already alluded to (see Section 3.2.3.1). The significant class A character of Cr(III), like that of Fe(III), signifies that the stability of its complexes increases in the order $F^- > Cl^- > Br^-$ and O-ligands > S-ligands (Earley and Cannon, 1965). Class B ions like Hg(II) have the opposite ligand preference (Nieboer and Richardson, 1980). Thus Cr(III) and Fe(III) often behave like Al(III), Ca(II), and Mg(II) which, in biological systems, predominently seek out charged oxygen centres (Nieboer and Richardson, 1980). The high affinity for the $OH^-$ and $PO_4^{3-}$ ions is consistent with this (Rollinson _et al_., 1967; Mertz, 1969).

From a biochemical viewpoint (see Section 3.3), the similarities and differences in atomic parameters between Cr(III) and Fe(III) are important. Chromium(III) exists exclusively with octahedral geometry (i.e., is surrounded by 6 donor atoms), while iron(III) complexes can be 4-, 5- or 6-coordinate. The octahedral Cr(III) and Fe(III) ions are of comparable size, with ionic radii of 76 picometers (pm) and 79 pm (high-spin state), respectively. These similarities in charge and size account for the observation that Cr(III) replaces Fe(III) in a number of biomolecules (see Sections 3.3.2 and 3.3.4.2).

### 3.2.3.3 Solubility of Chromium(III) Complexes

Chromium(III) oxide ($Cr_2O_3$), even when hydrated ($Cr_2O_3 \cdot xH_2O$), is insoluble in water. Chromium(III) phosphate complexes are also virtually insoluble. Anhydrous complexes of common anions have low water solubility (e.g., $CrBr_3$, $CrCl_3$, $Cr_2(SO_4)_3$)*, while corresponding hydrated forms have considerable water solubility (e.g., $[Cr(H_2O)_6]Br_3$, $[Cr(H_2O)_4Cl_2]Cl \cdot 2H_2O$, $Cr_2(SO_4)_3 \cdot 15H_2O$). Salts of acetate, oxalate and nitrate, which only occur in hydrated forms, are also soluble (Weast, 1973; IARC, 1980).

### 3.2.4 Chemistry of Less Stable Oxidation States

Judging by the lack of published documentation, industrial exposure to chromium(II) compounds appears not to be a serious concern. Anhydrous chromium(II) salts (e.g., chloride, acetate, formate, etc.) are relatively stable (Merck, 1983; Wasson, 1976). They are such powerful reducing agents (see Section 3.2.1) that they are oxidized to Cr(III) by water with the liberation of hydrogen gas ($H_2$). In addition, reaction with molecular oxygen ($O_2$) is rapid, and in fact chromium(II) solutions are convenient $O_2$-absorbants in the purification of laboratory gases. It is obvious that because of this reactivity, Cr(II) solutions must be maintained anaerobically. Under these conditions exposure to these strong irritants will be minimized.

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* Some of these chromic salts exist in two forms, a soluble and insoluble modification.

As already indicated in Section 3.2.1, Cr(V) and Cr(IV) are relatively unstable. Since very few complex compounds of chromium in these oxidation states have been isolated and characterized (Wasson, 1976; Cotton and Wilkinson, 1980,p.731), industrial exposure is unlikely. However, considerable evidence has accumulated that both Cr(V) and Cr(IV) are formed as transient intermediates in the reduction of Cr(VI) (i.e., chromates) to Cr(III). Of these intermediates, those of Cr(V) are somewhat more stable. The magnitude of the half-lives amounts to minutes, allowing their characterization by spectrometric techniques (Wiberg, 1965; Beattie and Haight, 1972; Wetterhahn Jennette, 1982). Because of the inherent instability of these oxidation states, it is conceivable that they are able to generate cytotoxic radicals such as those derived from peroxide (e.g., the hydroxyl radical, ·OH; superoxide, $O_2^-$). Active oxygen species of this type have been incriminated in tumour promotion (Marx, 1983).

Numerous organochromium compounds have been synthesized and characterized (Sneeden, 1975; Wasson, 1976). (Organometallic compounds contain a distinct metal-carbon bond.) In many of the compounds chromium has a low valency (-II,-I,0,+I), although derivatives of chromium(II), (III), and (IV) are also common. The majority of organochromium compounds are prepared under stringent conditions: in organic solvents, and under anaerobic and anhydrous conditions. Many of the substances are insoluble in water and are air and moisture sensitive; although a small number are water soluble and stable in aqueous media. Chromium hexacarbonyl ($Cr(CO)_6$) is considerably less volatile than nickel carbonyl ($Ni(CO)_4$) and iron pentacarbonyl ($Fe(CO)_5$), even though it sublimes at room temperature (Wender and Pino, 1968; King, 1976; Stokinger, 1981). Brief et al. (1971) suggest that because of this low volatility chromium carbonyl poses less of an occupational hazard than its nickel and iron counterparts. Although metal carbonyls are used in the preparation of metals of high purity, as catalysts for organic reactions, and as gasoline additives, the specific industrial use of chromium carbonyl appears to be low compared to those of manganese, iron, cobalt and nickel (Wender and Pino, 1968; Brief et al., 1971; Merck, 1983; Stokinger, 1981). An $LD_{50}$

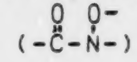
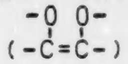
in mice of 30 mg $kg^{-1}$(i.v.) has been reported (RTECS, 1977). This compares to about 50 mg $kg^{-1}$(i.v.) for nickel carbonyl (RTECS, 1977; Stokinger, 1981).

## 3.3 Chromium Biochemistry

### 3.3.1 Introductory Remarks

It may be said with some certainty that chromium is one of those elements of which trace levels are required, but when accumulated in excess becomes toxic. Because it is an essential metal in animals, metabolic pathways for chromium must have evolved. In this section, a brief description is given of what is known about its interaction with low-molecular-weight biomolecules, proteins and polynucleotides, and about its biological function. The coverage is intended to provide background appropriate to our treatment of the absorption/distribution/excretion of chromium (Section 3.4), and to the development of chromium toxicity models (Section 3.6).

### 3.3.2 Interaction with Low-Molecular-Weight Biomolecules

The strong affinity of Cr(III) for charged oxygen centres has already been mentioned (Section 3.2.3.2). Complexes of stability comparable to that recorded for Fe(III) are known; for example, with the following ligands: hydroxide, phosphate, acetate, oxalate, malonate, phenolate, the iron chelating agents (the siderophores or siderochromes) involving the hydroxamato  $(-\overset{\overset{O}{\|}}{C}-\overset{\overset{O^-}{|}}{N}-)$ and catecholato  $(-\overset{\overset{-O}{|}}{C}=\overset{\overset{O^-}{|}}{C}-)$ functional groups (Martell and Smith, 1977; Smith and Martell, 1976). There is now general agreement that siderochromes are responsible for microbial iron transport and perhaps for the solubilization of extracellular iron (Neilands, 1979; Hughes, 1981). The Cr(III) analogues are also known to be taken up by cell suspensions, and are thus biologically active (Emery, 1978). Other ligands are known to affect the transport of Cr(III) across absorptive tissue. Oxalate administered concurrently with Cr(III) enhanced this ion's transport through rat intestine in both *in vitro* and *in vivo* (oral feeding) experiments. This ligand not only enhanced absorption, but also increased urinary excretion of chromium. Phytate (inositol hexaphosphate, a fully phosphorylated six-carbon cyclic polyhydric alcohol) reduced both transport and absorption, while EDTA and citrate had little effect (Chen *et al*., 1973). No doubt these ligand effects are the result of complex formation and is related to the permeability of the resulting Cr(III) complexes (i.e., their molecular weight, charge, and lipid solubility). Complex formation is also known to enhance or prevent the polymerization of Cr(III) that accompanies hydrolysis. Rollinson *et al*. (1967) have demonstrated that hydrolysis in the presence of phosphate at pH 7.0 (Cr(III), $10^{-4}$ M; $HPO_4^{2-}$, 0.02 M) was prevented by pyrophosphate ($10^{-3}$ M), and enhanced by oleate ($10^{-3}$ M). EDTA and citrate also have such inhibitive capability (Sanderson, 1976,1982). This was tested by dialysis, and thus by the ability to diffuse as low-molecular-weight species across a semi-permeable membrane (4.8 nm pore size).

Cr(III) is known to form complexes with amino acids. Those of aspartic acid and glutamic acid have been characterized (Evans _et al._, 1979), and crystallographic data are available for $Na[Cr(L\text{-}cys)_2]\cdot 2H_2O$, $Cr(gly)_3$, and Cr(L-his)(D-pen) (with L-cys denoting L-cysteine; gly, glycine; L-his, L-histidine; and D-pen, D-penicillamine (β,β-dimethylcysteine)). As expected for a borderline metal ion, these amino acid ligands are attached to Cr(III) through the N atom of the α-amino group and an O atom of the α-carboxylate, as well as through the S atom of the side-chain sulphydryls (L-cys, D-pen), the imidazole heterocyclic N of histidine, and presumably the O atom of the β-carboxylate of aspartic acid (de Meester _et al._, 1977; de Meester and Hodgson, 1977; Evans _et al._, 1979; Gergeley and Sóvágó, 1979; Connett and Wetterhahn, 1983). The class A character of Cr(III) is characterized by the $Cr(L\text{-}cys)_2^-$ complex anion. In acid solution this anion is subject to acid hydrolysis, which cleaves the Cr(III)-S bond and replaces it with water (the α-amino and α-carboxylate groups remain attached) (Connett and Wetterhahn, 1983). By contrast, metal-sulphur bonds of class B ions such as Hg(II) are extremely stable, even in very acid solution (Section 3.2.3.2; Nieboer and Richardson, 1980).

The oxygen-seeking ability that characterizes chromium is specifically highlighted in its interaction with nucleotides. Unlike borderline metal ions with considerable class B character (e.g., Cu(II), Cd(II)) and class B ions (e.g., Hg(II), Pd(II)), Cr(III) does not exhibit much affinity for the N-donor centres provided by the nucleotide bases (Martin and Mariam, 1979; Spiro, 1980). Instead it is attached through the phosphate moieties of the nucleotide molecules (DePamphilis and Cleland, 1973; Legg, 1978), and in this it resembles Mg(II) and Ca(II). As might be expected from our previous discussion in Section 3.2.3.1, the Cr(III)-nucleotide complexes are kinetically inert, and this appears to be of biological consequence in relation to Mg(II) biochemistry. Mg(II) plays a major role in living organisms because it is a cofactor in the production, storage and use of the high energy compound ATP; and because it is essential for the stabilization of polynucleotides, such as DNA and RNA, by acting as a counterion for the negatively charged phosphate moieties in these macromolecules. As explained more fully in Section 3.3.7, the replacement of Mg(II) by Cr(III) usually results in inhibition of critical enzymes, and furthermore may be expected to alter the three-dimensional geometry of DNA and RNA so essential to their role in genetic processes. And finally, Cr(III) has considerably greater affinity for N-containing ligands than Mg(II) (e.g., Warren _et_ _al_., 1981), which opens up additional modes of interaction with biomolecules which potentially increases its toxicity.

In Section 3.2.2.4 the chemical reactivity of the chromate ion was discussed. Because this Cr(VI) species is an anion, the central Cr(VI) ion does not interact directly with biological ligands at physiological pH. However because chromate can oxidize intracellular reductants, the generated Cr(III) ion is available for such complex-formation reactions, often with unreacted substrate. At physiological pH, chromate attacks most low-molecular-weight intracellular reducing agents. Of these, thiol-containing compounds are most prominent and include cysteine, glutathione, and lipoic acid. They are oxidized to the corresponding disulphide (Gergely and Sóvágó, 1979; Rabenstein et al., 1979; Connett and Wetterhahn, 1983). Although Samitz and Katz (1964) have reported that methionine was oxidized by chromate at pH 7, more recent work has shown this reaction to be slow compared to thiols (Connett and Wetterhahn, 1983). Ascorbic acid and lactic acid are also capable of reducing chromate at physiological pH values (Samitz and Katz, 1964; Connett and Wetterhahn, 1983). In fact, chromate is potentially capable of oxidizing most intracellular reducing agents because of its significant positive formal potential ($E^{o'}$) at physiological pH (see Section 3.2.1). Other possibilities include the reduced forms of nicotinamide adenine dinucleotide, flavin adenine dinucleotide, and coenzyme Q (a quinone) all of which have

$E^{o'}$ values less positive than chromate (Connett and Wetterhahn, 1983). Since a number of these reductants are present in significant concentrations in cells, the chromate ion will eventually be reduced. The magnitude of the biological half-life of chromate is predicted to be minutes to hours (see Section 3.2.2.4).

### 3.3.3 Interaction with Polynucleotides

One of the earliest indications that chromium can interact with nucleic acids was reported by Herrmann and Speck (1954). They observed that chromate-treated tissue homogenates decreased the extractability of nucleic acid into hot trichloroacetic acid. These authors correctly concluded (based on solution colour changes) that chromate is reduced to Cr(III) by tissues (see Section 3.3.6), and that the reduced form interacted with the nucleic acids. Laboratory studies have indicated that Cr(III) binds to nucleic acids, and most likely is bound to exterior phosphate groups (Eisinger et al., 1962; Danchin, 1975). Cr(III):DNA-P ratios up to 1 have been observed (Okada et al., 1981). Furthermore, Cr(III) has a tendency to precipitate polynucleotides and, like Ni(II) and Zn(II), increases their thermal stability (Fuwa et al., 1960). Significant amounts of chromium (20-400 $\mu g\ g^{-1}$ of RNA or DNA) have been found in nucleic acids from a number of sources (Wacker and Vallee, 1959). Ono et al. (1981) examined the distribution of several metals in nuclei and nucleoli from rats fed a conventional diet containing 0.3 $\mu g\ g^{-1}$ of Cr.

They found that, in contrast to Ca, Zn, Cu, and Mn, the ratio of Ni and Cr in nuclei to that of whole cells was relatively high (20% compared to 1 to 3%). Concentrations of Ni and Cr in nucleoli (relative to the nuclear content) were considerably enhanced compared to the other metals, and were more resistant to treatment with nucleases. Additional evidence that Cr(III) accumulates in the nucleus because of its high affinity for nucleic acids may be derived from cell culture studies. Levis et al. (1978a,b) observed that DNA isolated from BHK fibroblasts treated with potassium dichromate contained large amounts ( > 1%) of Cr measured as Cr(III). Chromate-induced cellular DNA-protein cross-links have been observed in vivo and in vitro and attest further to the strong interaction of Cr(III) with DNA and other polynucleotides (see Section 3.3.4.1).

### 3.3.4 Interaction with Proteins and Enzymes

#### 3.3.4.1 Cr(III) Cross-Links

Because of its application in the tanning of leather, the interaction of Cr(III) with collagen is undoubtedly the most comprehensively studied reaction with proteins. Chemical modification studies have revealed that Cr(III) cross-links predominantly involve the carboxylate groups of the polypeptide chains of collagen, although the polymerization of the hydrated Cr(III) ion by hydroxide ion is also required (Gustavson, 1958; Kühn and Gebhardt, 1960; Mertz, 1969). Other protein systems have also been examined. Compared to Al(III) and Fe(III), the dimerization of conarachin II (a groundnut protein) at pH 5.3 occurred more slowly with Cr(III), and the product was more stable to dialysis against phosphate buffer (Naismith, 1958). The dialysis step reversed the Al(III)- and Fe(III)-induced dimerization by removal of the metal ion. Cr(III) salts have produced cross-linking between DNA and protein *in vitro*, when the constituents were mixed in solution or when isolated nuclei were treated with Cr(III). Hexavalent chromate was ineffective in these experiments (Fornace *et al*., 1981). However, chromate-treated rats (Tsapakos *et al*., 1981, 1983) and chromate-treated cultured mammalian cells (Fornace *et al*., 1981) were found to have similar cellular DNA-protein cross-links. It is now widely accepted that chromate is reduced intracellularly to Cr(III) (see Section 3.3.6). Fornace *et al*. (1981) suggest that *in vivo*, the linking of DNA and protein or perhaps other macromolecules by the Cr(III) ion may affect polymerase activity during both DNA replication and repair, with resultant mutagenic and carcinogenic consequences (see Section 3.6).

### 3.3.4.2 Cr(III)-Binding and its Consequences

There is solid evidence that Cr(III) binds to the Fe(III)-transport protein transferrin. The bulk of the Cr(III) administered to rats and occurring in serum is bound to transferrin, while secondary binding occurs to other proteins (e.g., albumin) (Hopkins and Schwarz, 1964;

Jett et al., 1968). In vitro studies have shown that Cr(III) competes for the same binding sites of transferrin as Fe(III) (Aisen et al., 1969; Harris, 1977; Emery, 1978). Since iron rarely occupies more than a third of the available binding sites in transferrin, the small amount of plasma chromium ($\sim$1 μg $l^{-1}$) can be easily accommodated. It is considered that transferrin is the main serum transport protein for this ion. Serum transferrin carries iron from the breakdown sites of haemoglobin in the reticuloendothelial cells of spleen and liver back to the sites in bone marrow for the synthesis of haemoglobin (Hughes, 1981). Interestingly, the intravenous administration of Cr(III) in humans has established that Cr(III) is initially localized primarily in the liver, spleen, body soft tissues and bone (Lim et al., 1983). Studies with reticulocytes have demonstrated that transferrin is transported into cells by endocytosis (Light and Morgan, 1982). In addition, transferrin is involved in the transport of iron to cells following the ferritin-mediated absorption of iron from the gastrointestinal tract. As for reticulocytes, the transferrin uptake by other cells, and thus iron uptake, also appears to occur by endocytosis (Emery, 1978). The possibility exists, therefore, that Cr(III) may be taken up in a similar manner.

In vitro experiments indicate that Cr(III) on binding to enzymes is accompanied by the enhancement or inhibition of catalytic activity. Cr(III) is known to restore the activity of apocarboxypeptidase (Vallee *et al.*, 1958). This demonstrates that Cr(III) can replace Zn(II), since carboxypeptidase is a zinc metalloenzyme. Cr(III) also satisfies the second-metal requirement of phosphoglucomutase. The greatest activity of this enzyme occurs in the presence of three activators: hexosediphosphate, Mg(II), and a second metal (Stickland, 1949). Consideration of affinities and the relative concentrations required suggest that if this two-metal activation has any physiological analogue, then Cr(III) and Mg(II) are the metals concerned. Concentrations as low as $10^{-6}$ M of Cr(III) were effective. In *in vitro* experiments, non-physiological concentrations of the metal ion are often required, making extrapolation to *in vivo* conditions difficult. Nevertheless, these type of experiments often demonstrate potential effects. This is illustrated by a recent example involving the alteration by Cr(III) of ribonucleic acid synthesis directed by DNA and chromatin isolated from mouse liver. Maximum enhancement (seven-fold) occurred when Cr(III) was pre-incubated with DNA or chromatin with a Cr(III):DNA-P molar ratio near 1 (Okada *et al.*, 1981, 1982). Although such high Cr(III):phosphate ratios are unlikely to occur *in vivo*, subsequent experiments with mice demonstrated that the enhancement did occur *in vivo* after intraperitoneal administration of chromium(III) chloride ($\geq$ 0.05 mg Cr/kg) (Okada *et al.*, 1983).

Cr(III) has been shown to inhibit blood clotting *in vitro* (Chargaff and Green, 1948). More recently, selective inhibition of the electron transfer functions of cytochrome C and plastocyanin have also been reported (Connett and Wetterhahn, 1983). Interestingly, the large fraction of erythrocyte chromium derived from chromate uptake is bound to haemoglobin, although its effect on oxygen binding has not been demonstrated (Kitagawa *et al*., 1982; Sanderson, 1982). In a detailed study of soluble skin proteins, Samitz *et al*. (1969) illustrated that more chromium was accumulated (20-fold) from Cr(III) sulphate solutions than from equimolar potassium dichromate solutions. Blocking of amino groups by acetylation and carboxylate groups by methylation decreased the binding of chromium. The decrease was much more marked for the latter, strongly suggesting again the prominence of carboxylate groups in the interaction of Cr(III) with protein. As discussed in Section 3.6.3, such interactions may be significant in the etiology of chromium dermatitis.

Inhibition with potentially serious physiological consequences involves the replacement of Mg(II) (and perhaps Mn(II) in those instances where it is the native ion; Ochiai, 1977) in reactions where ATP, ADP or similar nucleotides are substrates. Cr(III) forms non-labile complexes with these mononucleotides (see Section 3.3.2). The Cr(III) nucleoside di- and triphosphates inhibit kinases (which involve the transfer of phosphate from ATP to a substrate; DePamphilis and Cleland, 1973; Janson and Cleland, 1974; Gupta et al., 1976), pyruvate carboxylase (Armbruster and Rudolph, 1976), and ATPases (Schuster et al., 1975). For example, Cr(III) inhibition of Mg(II)-ATPase activity has been reported for plasma-membranes of intact hamster fibroblasts in culture (Luciani et al., 1979). Inhibition in this system of nucleoside permeases has also been observed (Levis et al., 1978b).

And finally when protein systems become saturated with Cr(III), denaturation occurs (Clark, 1959). Bianchi et al. (1979) postulate that denaturation of membrane structures may contribute to the cytotoxicity of Cr(III).

### 3.3.4.3 Chromate as Oxidant and Substrate

Connett and Wetterhahn (1983) summarized the evidence for the conclusion that not many purified proteins reduce chromate at physiological pH values. However, this statement needs to be qualified because the analytical procedures employed often precluded the detection of the chromate consumed by the sulphydryl-equivalents available in the proteins studied. Unequivocal evidence for the reduction of chromate by haemoglobin is available (Samitz and Katz, 1964; Connett and Wetterhahn, 1983). The resultant Cr(III) appears to be bound to haemoglobin (Gray and Sterling, 1950; Kitagawa et al., 1982). Glutathione reductase is also inhibited by chromate, and it is surmised that it involves the oxidation of the active site sulphydryl groups (Connett and Wetterhahn, 1983). In vitro studies with mitochondria have shown that respiratory dehydrogenases are also inactivated (Broughall and Reid, 1974). Since sulphydryl groups are critical to the catalytic function of a number of other enzymes (e.g., δ-aminolevulinic dehydrase, urease, adenylate kinase), chromate oxidation of such enzymes should be investigated. Preliminary inhibition data for urease have indeed been reported (Henry and Smith, 1946).

Evidence is mounting that chromate is reduced by microsomal electron-transport cytochrome P-450 systems. The slow rate of reduction in control samples (microsomes without NADPH; NADPH in the absence of microsomes), and the inhibition by P-450-specific inhibitors confirm that this reaction is enzymatic (Gruber and Jennette, 1978; Garcia and Wetterhahn Jennette, 1981). A practical application of this observation is the metabolic deactivation of chromate mutagenicity in the Salmonella-microsome test. The resultant Cr(III) is non-mutagenic (Petrilli and De Flora, 1978; De Flora, 1978; Levis and Bianchi, 1982). An interesting development of note in relation to the microsomal reduction of Cr(VI) is the spectrometric characterization of relatively long-lived (hours) Cr(V) intermediates (Polnaszek, 1981; Wetterhahn Jennette, 1982). Goodgame et al. (1982) were able to generate Cr(V) in the absence of microsomes, when chromate was mixed with ribonucleotides. Interestingly, the reaction did not occur with the corresponding deoxyribonucleotides, suggesting that the oxidation of the ribose ring is involved. The toxicological implication of unstable Cr(V) intermediates has already been addressed (Section 3.2.4.).

### 3.3.5 Cellular Association and Uptake

Numerous cell-culture studies have shown that cellular uptake of chromate is at least ten times greater than that of Cr(III) from equimolar solutions (Gray and Sterling, 1950; Levis *et al.*, 1978b). Speciation studies have revealed that intracellular reduction of chromate to Cr(III) occurs (Levis *et al.*, 1978a,b; Kitagawa *et al.*, 1982; Levis and Bianchi, 1982). At least in the case of erythrocytes, the cell-associated chromium is primarily cytosolic. Since the resultant Cr(III) has a high affinity for intracellular binding sites (see previous discussions in Sections 3.3.2 to 3.3.4), it is not surprising that little of the chromate accumulated by cells can be removed by washing with isotonic buffer (e.g., 6% at t=0 and 1% at t=6 h after exposure to chromate; Kitagawa *et al.*, 1982). The sensitivity of chromate uptake by erythrocytes to stilbene inhibitors suggests that it utilizes an anion channel by analogy with sulphate and phosphate uptake (Kitagawa *et al.*, 1982). On the other hand, measurement of activation energies and other uptake characteristics implicate diffusion-controlled uptake (Ormos and Mányai, 1974). In agreement with this are the findings of Sanderson (1976), who showed that metabolic inhibitors did not inhibit uptake by mastocytoma cells.

It appears that chromate uptake is not a prerequisite for all cytotoxic effects. Studies with intact cultured hamster fibroblasts have demonstrated that chromate, on initial exposure, enhances the cell membrane permeability to nucleosides. This is followed by a second phase in which inhibition of uptake occurs. Structural modification of cell membranes, presumably by the oxidative action of chromate, is believed to stimulate simple inward diffusion of the nucleosides (Bianchi et al., 1979). Subsequently, when Cr(III) accumulates at the membrane surface, nucleoside permeases are thought to be inactivated and perhaps even denatured. Separate administration of Cr(III) salts do indeed inhibit nucleoside uptake (Levis et al., 1978b).

### 3.3.6. Interaction with Tissues

Chapter 5 in this report deals with the hypersensitivity responses to chromium compounds. Allergic contact dermatitis is the most prominent reaction. Epicutaneous applications (patch tests) and percutaneous absorption measurements have shown the importance of valency, type of salt, pH, and such factors as the presence of reducing agents (see Chapter 5). In this section, we will briefly outline some basic concepts that help to rationalize the often conflicting results reported for the transport of chromium compounds into and through the skin. Comprehensive reviews summarizing the empirical data are provided by Pederson (1982) and Polak (1983), and of course in Chapter 5 of the present document. In brief, the major findings are as follows:

1. Cr(VI) compounds penetrate the skin more readily than Cr(III) compounds.
2. The penetration of chromate increases with increasing pH.
3. The penetration of Cr(III) salts depends on the nature of the anion.
4. The difference in penetration of Cr(VI) and Cr(III) compounds appears to be governed by the epidermal layer which forms the main barrier to uptake.
5. Skin and skin components reduce chromate.
6. And finally, Cr(III) binds strongly to skin and skin proteins.

Before these results are interpreted and rationalized, a brief review of the structure and composition of human skin is presented.

A cross section of human skin is depicted in Fig. 3.4. The first phase of percutaneous absorption is the diffusion of the toxicant through the epidermis (Klaassen, 1980). The stratum corneum comprises a dead surface layer of the skin, and movement through it is by passive diffusion. Water soluble (polar) substances appear to diffuse through the hydrated component of the stratum corneum; while non-polar molecules dissolve in and diffuse through the lipid matrix situated between the protein filaments which make up this layer. Subsequent to the epidermis, the toxicant passes through the dermis which corresponds to a porous, non-selective aqueous diffusion medium. Movement is again by simple diffusion and the toxicant now has easy access to the systemic circulation (see Fig. 3.4). Several additional points need to be made. Because it acts as a barrier, the removal of the stratum corneum (e.g., by abrasion, injurious agents, such as acids and bases, and burns) causes an abrupt increase in the permeability of the epidermis. Hydration of the stratum corneum is also known to enhance absorption, as does the removal of its lipid components on contact with organic solvents. Furthermore, penetration increases with the diffusivity and thickness of the stratum corneum. The hair follicle also appears to be a route of entry. And finally, a guideline to the composition of the stratum corneum is as follows: 52 - 55% water-insoluble material (crude keratin), 5 - 10% water, 19 - 27% water-soluble material, and 10 - 22% ether soluble constituents. The water-soluble materials consists of 40% free amino acids, 10% carbohydrates, 1% citric acid, 12% lactic acid among other constituents (Flesch, 1958; Samitz and Katz, 1964).

There is considerable evidence that the chromate ion diffuses freely through the epidermis while the movement of the Cr(III) ion is inhibited (Mali _et_ _al_., 1963). The diffusion of the Cr(III) ion is retarded for a number of reasons. First of all, Cr(III), in contrast to chromate, binds strongly with dermal tissue and its low-molecular-weight constituents (Mali _et_ _al_., 1963;

Samitz and Katz, 1963,1964; Samitz et al., 1969). Secondly, the species present in solution is highly dependent on the Cr(III) salt dissolved. For example, solutions of $[Cr(H_2O)_6]Cl_3$, like those of $KCr(SO_4)_2 \cdot 12H_2O$, generate the fully hydrated ion, $Cr(H_2O)_6^{3+}$, while the normal commercially available salt $[CrCl_2(H_2O)_4]Cl \cdot 2H_2O$ yields the singly-charged ion $CrCl_2(H_2O)_4^{+}$. The net charge on the Cr(III) species influences both the diffusion rate and the ability to bind to tissues. The degree of hydrolysis, and thus polymerization with a concomitant reduction in diffusion, is also highly dependent on the Cr(III) salt selected. Of course, the latter process increases with increasing pH of the vehicle. These differences adequately explain that undamaged human skin is poorly penetrated by the commercially available sulphate and nitrate of Cr(III), whereas the chloride penetrates almost as well as potassium dichromate (Polak, 1983).

The observation that the diffusion of chromate increases with pH concurs with the concomitant decrease in its oxidizing power (see Sections 3.2.1 and 3.2.2.4). Consequently, less of it is converted to Cr(III) because of reaction with skin proteins and water soluble constituents (i.e., lactic acid, amino acids; see Section 3.3.2).

And finally, the observation that the removal or damage of the stratum corneum permits the uptake of Cr(III) salts may have serious toxicological consequences. In a recent case report, significant quantities of chromium were absorbed by a man who became accidentally immersed in hot acidic Cr(III) sulphate solution (Kelly et al., 1982). Although no oral ingestion of chromium occurred, the chromium content of the tissues at necropsy was severely elevated as was that found in blood and urine before death. Burnt skin also accumulated extremely high Cr levels. The clinical course was dominated by burns, intravascular haemolysis and acute renal failure. Other cases of severe nephritis resulting from the cutaneous absorption of chromate are known (see references in: Stokinger, 1981; Kelly et al., 1982). Severe kidney toxicity has also been observed in acute chromate poisoning (Goldman and Karotkin, 1935; Sharma et al., 1978; Kelly et al., 1982).

### 3.3.7 Biological Functions

In Section 3.3.4.2 we reviewed the consequence of Cr(III) binding to enzymes. Both inhibition and stimulation of catalysis occurred. Although the exact physiological importance of this type of role is hard to assess, it illustrates the potential of Cr(III) to regulate and alter physiological processes. This is most vividly illustrated by the discovery of the chromium glucose tolerance factor (CrGTF). A brief summary of these developments are provided in this section.

There is no doubt that chromium is an essential metal since its deficiency has serious consequences in man and other animals. Signs of chromium deficiency in man include glucose intolerance, elevated circulating insulin, elevated serum cholesterol and triglycerides, and peripheral neuropathy and metabolic encephalopathy (Jeejeebhoy _et al_., 1977; Freund _et al_., 1979; Anderson, 1981). An increased risk of coronary disease also may occur (Mertz 1982b). The CrGTF has been identified in Brewers' yeast as a Cr(III) dinicotinic acid-glutathione complex (Mertz, 1975,1982a,1983). It possesses insulin potentiating capability presumably by forming a complex with insulin, which then acts at the membrane level as a co-hormone. Less insulin is required in the presence of the CrGTF. Quite a few Cr(III) complexes can mimic this species, and appear to be characterized by enhanced intestinal absorption as well as enhanced placental transfer (Mertz _et al_., 1974; Mertz, 1975; Anderson _et al_., 1980). GTF activity of Cr(III) compounds with non-physiological ligands has been demonstrated (Schwarz and Mertz, 1959). Potassium trioxalatochromium(III) is also active. It may be recalled that oxalate enhanced the gastrointestinal absorption of Cr(III) in rats (see Section 3.3.2), and as already indicated this is characteristic of GTF-active compounds. Synthetic analogues of the Brewers' yeast CrGTF are as active as the natural product. These compounds are prepared by refluxing a 1:2:2:1:1 mixture of Cr(III) sulphate, nicotinic acid, glycine, glutamic acid, and cysteine in aqueous ethanol (Mertz _et al_., 1974; Toepfer _et al_., 1977; Haylock _et al_., 1983). Since all of these ligands are physiological, it seems possible that GT factors can be synthesized in vivo from Cr(III), and that there are a number of active forms. There is some evidence which corroborates these speculations. Polansky and Anderson (1980) have shown that radiolabelled $^{51}CrCl_3$ and $Na^{51}CrO_4$ administered parenterally to rats shortly before birth, are transported across the placenta to the fetus. They concluded that either inorganic chromium crosses the placenta, or it is converted into a form that does. Similarly, Polansky et al. (1981, 1982; also see Anderson et al., 1982a, 1983a,b) in controlled chromium supplementation experiments of free-living human subjects found a significant reduction in glucose levels of subjects with somewhat elevated glucose. The chromium was administered orally as Cr(III) chloride or as brewers' yeast. Similar results have been found in other studies (Offenbacher and Pi-Sunyer, 1980; Mertz, 1982b; Mossop, 1983). An increase in the fraction of high density cholesterol lipoprotein was also noted. And finally, Gonzalez-Vergara et al. (1982) claim to have isolated a CrGTF factor from yeast that appeared to contain a tryptophan-like ligand, rather than nicotinic acid and glutathione.

The glucose tolerance factor illustrates again that when surrounded by the correct ligand environment, Cr(III) can be absorbed and transported. Recent mutagenicity tests have also been positive for singly-charged aromatic amine derivatives of Cr(III), suggesting cellular uptake by species of the type $Cr(A)_2X_2^+$ (Warren et al., 1981).

Consequently Cr(III) should no longer be considered as lacking the ability to be taken up by cells.

## 3.4 Absorption, Distribution and Excretion

### 3.4.1 Intake and Absorption

As for most toxicants there are three primary modes of chromium absorption: namely, via the airways, skin, and the gastrointestinal tract. We have already considered the dermal route (Section 3.3.6), and it is more appropriate to treat the uptake by the respiratory tract in relation to biological monitoring (Section 3.5). Gastrointestinal absorption will be considered in this section.

Chromium is said to be poorly absorbed, and often a figure of about 1 to 3% is quoted (e.g., Doisy *et al*., 1969; Underwood, 1977; Kumpulainen *et al*., 1979). However, and as already pointed out (Section 3.3.7), the uptake is dependent on the chemical form of the chromium. For example, preliminary animal experiments suggest that 10 - 25% of chromium in Brewers' yeast is absorbed by rats (Mertz *et al*., 1974; Underwood, 1977). Furthermore, Underwood (1977) points out that the presence of other metals such as zinc may have a marked effect, as is the case for the absorption of most metals. Rat studies indicate that fasting also enhances uptake (MacKenzie *et al*., 1959). Even though Cr(VI) compounds are somewhat better absorbed than those of Cr(III) (Donaldson and Barreras, 1966; Underwood, 1977), the difference is smaller than expected from the much greater ability of Cr(VI) to cross cell membranes (see Section 3.3.5). Donaldson and Barreras (1966) have unequivocally demonstrated that human gastric fluids effectively reduce chromate to Cr(III), and in this way absorption is diminished. Interestingly for human subjects (Donaldson and Barreras, 1966), absorption following duodenal administration showed that 6% of Cr(III) chloride was taken up (compared to 0.5% for oral administration), while about 40% of $Na_2CrO_4$ was absorbed after duodenal administration (compared to 10% on oral administration).

In a very careful study (from an analytical point of view), Kumpulainen _et al_. (1979) determined that in representative American diets the daily chromium intake was $62 \pm 28$ μg (high fat diets) and $89 \pm 56$ μg (low fat diets). Similar results have been reported in other studies (see Guthrie, 1982, for compilation and critical assessment). In relation to the chromium GTF function, a provisional recommendation for the dietary intake of trivalent chromium of 50 - 200 μg $d^{-1}$ has been made (Mertz, 1979,1980).

### 3.4.2 Distribution

The models depicted in Figs. 3.5 and 3.6 conveniently help to organize our discussion of the distribution and excretion of chromium. The individual functional compartments or components depicted in these figures are mostly based on animal studies, but the overall model was proposed and verified for the kinetics of Cr(III) in the human body (Lim et al., 1983). These workers studied the distribution and kinetics of intravenous $[^{51}Cr]$chromium(III) in six human subjects using a whole-body scintillation scanner, a whole-body counter, and plasma counting. Since chromium in the diet is most likely in the Cr(III) state, the models shown should reflect the normal chromium distribution patterns. The special cases of enteral and parenteral administration of chromates will be considered separately.

After absorption and release from the iron storage protein ferritin, Cr(III) is transported bound primarily to plasma transferrin (70-90%) (Hopkins and Schwarz, 1964; Jett et al., 1968; Sayato et al., 1980; all studies with rats). In man, as in animals, Cr(III) is localized primarily in the blood, liver, spleen, kidney, body soft tissues and bone as depicted in the accompanying models (Hopkins, 1965; Onkelinx, 1977; Jain et al., 1981; Langård, 1982; Lim et al., 1983). The pool of protein-bound chromium in the plasma presumably corresponds to transferrin. Long term storage appears to occur in certain tissues, especially in the liver and the spleen. This suggests that the deposition of chromium in these tissues is related to the function and processing of transferrin as this occurs in these organs. The work of MacKenzie et al. (1959) with rats has shown that very little Cr(III) administered by stomach tube, or introduced into the intestine, becomes associated with erythrocytes.

Distribution studies for the i.v. injection of $Na_2CrO_4$ in rats yield similar results to those discussed for Cr(III) (accumulation in blood, lungs, liver, kidney and spleen) (Langård, 1979). The work of MacKenzie _et al_. (1959) with rats has shown that oral (stomach tube) or intestinal administration of chromate results in the strong labelling of both erythrocytes and plasma. Sayato _et al_. (1980) illustrated that the bulk of erythrocyte chromium was bound to haemoglobin, as expected from _in vitro_ erythrocyte studies (see Section 3.3.5). Shih _et al_. (1972) have studied erythrocyte survival in man by injecting $^{51}Cr$-labelled erythrocytes. The half-life of the label, measured by urine excretion of $^{51}Cr$, was 50 - 60 days, while that of the erythrocytes was near 25 - 35 days. Presumably this observation is related to the chromium accumulation with time in rat spleen in Langård's sodium chromate work (Langård, 1979), since this organ disintegrates old red blood cells.

In applying the model in Fig. 3.6, accumulations from the central plasma compartment with short (in minutes) and intermediate (1-24 h) half-times ($t_{1/2}$) were simply considered as "uptake". Release from such "uptake" compartments occurred with slightly longer $t_{1/2}$ values ($\leq$2.2 d). Deposition in the "storage" compartments took considerably longer ($\sim$4 d), with very slow release ($t_{1/2}$=315 d). Interestingly, the "storage" compartments accumulated by far the most chromium (96%). Note that the kidney and lungs, which animal data suggest initially also accumulate Cr(III), are designated as "other organs" in Fig. 3.6 because these could not be resolved individually by the whole-body scan technique.

### 3.4.3 Excretion

In the model (see Figs. 3.5 and 3.6), two excretion pathways are noted; that cleared from the unbound (plasma) pool is excreted by the kidney with a half-time of 3.5 h, and that removed by desquamated cells and faeces with $t_{1/2}$ = 7 d (Lim et al., 1983). This dual excretion pathway is strongly supported by the animal data, even though the earlier conclusion that urinary excretion is the main route (Visek et al., 1953; Mertz, 1969) is often quoted without question. The animal data summarized by Langård (1982) and Sayato et al. (1980) clearly show that 5 - 20% of chromium parenterally administered as Cr(III) turns up in the faeces, while as much as 10 - 30% of the injected Cr(VI) is excreted in this way. It is interesting that in a study of welders exposed primarily to Cr(VI), Tossavainen et al. (1980) assigned a $t_{1/2}$ value of 15 - 41 h to the urinary excretion of chromium, which is comparable with the assignment of 2.2 d above for the efflux from the "uptake" compartments. Nomiyama et al. (1980) provide evidence for rabbits that chromium occurs in urine as Cr(III). Urine itself was also found to be reducing toward chromate. Furthermore erythrocyte-bound Cr(III), as encountered in exposure to chromate, appears to be an additional long-term storage pool, with a release time ($t_{1/2}$) of 50 - 60 d; see earlier comments in Section 3.4.2 on work by Shih et al. (1972).

In a recent report, Donaldson et al. (1982) conclude from serum ultrafiltration and urinary excretion studies of rats and dogs that "there may not be a strong reabsorptive mechanism for Cr(III) in the kidney, as has been previously reported". Collins et al. (1961) concluded that 63% of the glomerular filtrate was reabsorbed. Perhaps the data reported for dogs by these workers did not refer to an equilibrium situation, as the dialyzable fraction of Cr(III) in plasma was time-dependent (9% at 2.5 h and 3% at 10 h after injection). Onkelinx (1977) found that the ultrafilterable fraction of Cr(III) in rats remained constant for times $\geq 3$ h after intravenous injection (at $7 \pm 1.5\%$). Mutti et al. (1979) concluded from ultrafiltration studies on serum from welders that about 90% reabsorption occurred. They reported variable chromium clearance values (volume of plasma cleared per unit time of non-protein bound chromium), which is an unlikely situation and suggests that methodological errors occurred. Three lines of evidence indicate that the clearance of chromium in man is creatinine-like, and thus occurs by glomerular filtration without tubular reabsorption or excretion (Faulkner and King, 1976). Firstly, this is implied by the reduction in the variability of urinary chromium determinations when creatinine normalization or corrections are applied for differences in urinary flow rates (Section 3.5.3; Tola _et_ _al_., 1977; Rahkonen _et_ _al_., 1983). In a careful and definitive study of non-exposed individuals, Anderson _et_ _al_. (1982a) found that creatinine adjustment reduced the standard error by a factor of 2. Secondly, the linear relationships observed in man (non-exposed) between urinary chromium and urinary creatinine (Saner, 1980; Anderson _et_ _al_., 1983) denote that Cr(III) and creatinine have the same functional dependence on urinary flow rate, and thus by the basic mathematics of clearance (Faulkner and King, 1976; Nieboer _et_ _al_., 1983a), must have the same excretion mechanism. (Anderson _et_ _al_. (1982a) have indicated that Saner's chromium data are on the high side because of analytical problems; also see Section 3.5.2.) And finally, when the apparent percent reabsorption (Eqn 3.5) reported for controls and insulin-dependent diabetics

$$\%\ \text{Reabsorption} = \left[1 - \frac{\text{serum creatinine}}{\text{urine creatinine}} \cdot \frac{\text{urine chromium}}{\text{serum chromium}}\right] \quad (3.5)$$

by Vanderlinde _et_ _al_. (1979) is recalculated to incorporate the estimate that in man only 5 - 6% of the plasma chromium is available for glomerular filtration (Lim _et_ _al_., 1983), then 0% reabsorption results. (The analytical work in this study is of the highest quality.) Interestingly, Vanderlinde _et_ _al_. (1979) found urinary chromium to be 2-fold or more higher in insulin-dependent diabetics than in healthy normal individuals.

The implication of these conclusions about the renal clearance of chromium is discussed in our consideration of biological monitoring (Section 3.5.5.2).

## 3.5 Biological Monitoring

### 3.5.1 Status of Chromium Concentrations in Body Fluids and Tissues of Normal Adults

The general availability of sensitive instrumental methods for the routine analysis of elements at the ng $g^{-1}$ (ultratrace) and $\mu g\ g^{-1}$ (trace) levels in environmental and biological samples has revealed that contamination by containers, analytical reagents and airborne dust constitutes a serious source of both systematic and random errors. Interlaboratory comparisons (e.g., Parr, 1977; Brown et al., 1981; Sunderman, Jr. et al., 1982), and the use of standard reference materials (e.g., Parr, 1977; Kosta and Byrne, 1982) clearly show contamination as a limiting parameter in trace and ultratrace analyses (Kosta, 1982). This realization has resulted in severe downward adjustments in the normal levels of elements in human tissues (AIN, 1981) and body fluids (Versieck and Cornelis, 1980). Recent compilations of chromium levels in human tissues and body fluids provide a vivid example. Normal serum and urine chromium concentrations have decreased markedly with time; from $\sim 200\ \mu g\ l^{-1}$ to $0.1 - 0.2\ \mu g\ l^{-1}$, and from $\sim 150\ \mu g\ l^{-1}$ to $1\ \mu g\ l^{-1}$, respectively (Versieck and Cornelis, 1980; Guthrie, 1982). A graphical illustration of this trend is provided in Figs. 3.7 and

3.8. Gross contamination during collection, handling, storage and analysis can be incriminated (Versieck and Cornelis, 1980; Torgrimsen, 1982). Chromium, like nickel, is ubiquitous because of its presence in stainless steels. These uncertainties in normal body fluid levels have rendered it difficult to assess the nutritional role of chromium (the Cr/glucose/insulin inter-relationships; Veillon _et al_., 1982a), and to evaluate the extent of exposure in the workplace by biological monitoring. As with most other trace and ultratrace metals, the normal chromium concentrations reported for human tissues are likely in gross error because of extraneous additions during sampling and analysis (AIN, 1981; Guthrie, 1982). Recent developments in the analytical chemistry of chromium now permit the routine determination of nanogram quantities of this element in biological samples.

## 3.5.2 Technical Feasibility

### 3.5.2.1 Available Instrumental Methods

The extremely low natural levels of chromium in body fluids limits the number of analytical methods. For routine analysis, electrothermal atomic absorption spectrometry (EAAS) is the preferred technique. It is the least labour-intensive of the available methods, yet it exhibits suitable analytical sensitivity and precision. Techniques appropriate for intermethod comparisons are neutron activation and isotope dilution mass spectrometry. Because of phosphorus-32 interference with the analytical peak of chromium-51, chromium needs to be separated in neutron activation analysis from the irradiated sample (distilled as chromyl chloride; Versieck *et al*., 1978,1979). A similar complication occurs with the isotope dilution technique because a volatile chelate suitable for gas chromatographic processing needs to be isolated (Veillon *et al*., 1979). Because of elaborate sample treatment and handling, clean air facilities are required in both cases to prevent adventitious contamination. Another drawback circumventing routine use is that highly specialized instrumentation is required which is not generally available. Nevertheless, and as already indicated, both techniques serve as suitable references in intermethod comparisons (Harnly *et al*., 1983).

### 3.5.2.2 Contamination Control

In a recent review of contamination control in routine ultratrace analysis of toxic metals, Nieboer and Jusys (1983) suggested that the rigorous application of measures 1 to 9 in Table 3.3 will assure adequate blank levels and acceptable precision in most situations. However, they emphasize that if ultratrace analyses are to be carried out in a laboratory known to be subject to ambient metal fallout, such as is often encountered in industry, clean-air facilities are warranted. In their review these authors consider the major sources of contamination, recommend a systematic approach to dealing with and evaluating adventitious additions, and provide appropriate references for cleaning laboratory wares and other decontamination procedures. In the approach recommended by Nieboer and Jusys (1983) for the avoidance of contamination, the sophistication of the control measures required are linked to analytical need.

### 3.5.2.3 Determination of Chromium in Urine by EAAS

There is no doubt that the main stumbling block to reliable chromium determinations is contamination control. The direct analysis of neat urine is the most common practice, and this avoids many of the hindrances to the attainment of low blank values (c.f., measures 6-9 in Table 3.3). Proper control of contamination is readily achieved when collection and storage procedures known to minimize extraneous additions are employed, including the use of thoroughly acid-washed containers and other laboratory utensils (measures 1-5 in Table 3.3; Brown *et al*., 1981; Nieboer and Jusys, 1983; Nieboer *et al*., 1983b). In addition, some technical complications in the EAAS determination step are also evident (see Torgrimsen, 1982, for review). In the determination of normal urinary chromium levels, uncompensated non-specific spectrometer absorption (uncorrected background) no doubt has contributed to artificially elevated concentrations (Guthrie *et al*., 1978; Veillon *et al*., 1982a,b). These difficulties arise due to the complexity of the urine medium matrix (Harnly and O'Haver, 1977), and are accentuated by the need to operate the spectrometer near the detection limit. Nise and Vesterberg (1979) clearly illustrate that for elevated chromium levels (such as observed for industrially exposed individuals), this technical difficulty is less important. A perusal of available reports makes it clear that the use of modern spectrometers virtually eliminates the background correction problem (Veillon *et al*., 1982a,b; Anderson and Bryden, 1983), and circumvents the need to modify commercially available equipment (Harnly and O'Haver, 1977; Kayne *et al*., 1978; Kumpulainen *et al*., 1979). Nevertheless, the method of standard additions is still recommended. Our recent critical evaluation of the direct determination of nickel in urine (at the $\mu g l^{-1}$ level) illustrates that matrix difficulties can be minimized by dilution, providing significant levels of nickel occur in the sample (Nieboer *et al*., 1983a). Because background interferences are highly dependent on the spectrometer and graphite furnace characteristics, it behooves the analyst to assess quantitatively its dependence on such factors as temperature programming (Guthrie *et al*., 1978; Nise and Vesterberg, 1979; Veillon *et al*., 1982b). For example, Schaller *et al*. (1973) used a charring temperature of 1600° C, which according to Veillon *et al*. (1982b) causes premature loss of chromium before atomization, while Nise and Vesterberg (1979) found only a small dissipation at this temperature.

### 3.5.2.4 Determination of Chromium in Serum by EAAS

Although serum chromium concentrations have not been extensively employed to assess exposure, their determination is technically feasible. To date only the results obtained by neutron activation and EAAS have yielded reliable values (Versieck and Cornelis, 1980). As already indicated in Section 3.5.2.1, EAAS is most suitable for routine work. It is now widely accepted that almost all of the published normal serum chromium levels are in error, as in the case of urinary chromium (see earlier comments and Figs. 3.7 and 3.8). Two main reasons have been postulated to explain the observed discrepancies: namely, volatilization during sample drying and/or dry ashing, and contamination during sample collection and handling (Versieck and Cornelis, 1980). Considerable evidence is now available that demonstrates convincingly that losses during ashing procedures do not occur (Shapcott, 1979; Versieck _et_ _al_., 1978,1979; Versieck and Cornelis, 1980), leaving systematic and random errors due to contamination in explanation of the divergent reports. Versieck _et_ _al_. (1982) have demonstrated that stainless steel syringe needles contribute significantly to the chromium concentration in blood samples. Consequently, the IUPAC-recommended blood collection procedure for nickel with a Teflon intravenous catheter needle seems prudent (Brown _et_ _al_., 1981).

Dry ashing (Versieck _et_ _al_., 1978) or wet digestion (Kayne _et_ _al_., 1978; Vanderlinde _et_ _al_., 1979) techniques have proven satisfactory in the oxidative destruction of the serum matrix. It is our experience that dry ashing is more susceptible to extraneous additions or losses (by adsorption). The wet digestion techniques inherently involve less handling and minimize the surface area contact of the sample with containers and ambient air (see Table 3.3). Low temperature (overnight at 80° C) $HNO_3/H_2O_2$ digestion has been employed sucessfully. The chromium in the digested mixture is then determined after a five-fold dilution with ultrapure water (Kayne _et al_., 1978; Vanderlinde _et al_., 1979). Direct serum analysis after 1:1 dilution with $H_2O$ also appears feasible (Kumpulainen _et al_., 1983).

The reliability and performance of methods for the determination of chromium in whole blood have not been extensively evaluated (e.g., Rahkonen _et al_., 1983).

### 3.5.2.5 Determination of Chromium in Tissues

In the regulatory framework, the measurement of chromium in tissues is not an option, and thus a detailed review of the techniques employed is not warranted in the present context. Neither has the chromium content of hair been employed extensively as an index of industrial exposure (see Section 3.5.4). Nevertheless, several comments in relation to the interpretation of the chromium content of autopsy samples are pertinent. Firstly, the lack of a reliable data base on the mineral content of human tissues (see Sections 3.5.1 & 3.5.4) is not a serious problem for assessing the accumulation of chromium in exposed organs such as the lung, small intestine and skin, but may well prevent the detection of marginal elevation in other organs. Secondly, the absence of evidence that rigorous protocols were followed to eliminate contamination during collection, handling and analysis will again affect the determination with confidence of accumulations of chromium in organs not exposed to exterior factors.

### 3.5.3 Chromium in Body Fluids of Exposed Individuals

Alsbirk _et_ _al_. (1981), have employed serum chromium quite convincingly as an index of exposure. These authors found for welders, that serum chromium increased from ∿0.3 μg $l^{-1}$ to values near 2 μg $l^{-1}$ during 2.5 days of work. More recently, Rahkonen _et_ _al_.(1983) have compared the daily mean increase in chromium levels in whole blood, plasma and urine of manual stainless steel welders. Although all three concentrations correlated with ambient chromium [ as total Cr or Cr(VI)], the most significant relationship was found for whole blood (see Section 3.5.5.3 for further comments). Unfortunately, these authors do not elaborate on the analytical methods employed for blood and serum, nor on their reliability and performance. Other than that the levels reported for non-exposed individuals are on the high side, the data presented by them appear to be reasonable. Another report of average plasma levels of 35 ± 20 μg $l^{-1}$ for chromium manufacturing plant workers seems suspect (Wieser _et_ _al_., 1982). These extremely high concentrations are suggestive of contamination errors, and need confirmation. Although some earlier reports of plasma and whole blood chromium for workers exist (Mancuso, 1951; Gafafer, 1953; Baetjer *et al*., 1959), the studies were completed prior to the awareness of the devastating effects of contamination on ultratrace analysis. Because of limited data, the present discussion is therefore necessarily limited to urinary chromium as an index of exposure.

Typical concentrations of chromium observed in urine for exposed individuals are depicted in Figs. 3.9 and 3.11, where they are plotted as a function of water-soluble chromium in the working environment. It can be seen that values up to 150 $\mu g\ l^{-1}$ have been reported. The parameters describing the relationships between urinary and ambient chromium concentrations for welders are summarized in Table 3.4.

Several conclusions may be derived from the data depicted in Figs. 3.9 - 3.11 and compiled in Table 3.4. First of all, a substantial fraction of the ambient chromium in manual metal arc welding of stainless steel is present as water-soluble Cr(VI), in agreement with Stern's recent conclusions. Stern (1982) also points out that welding fume is almost entirely respirable. Consequently, it may be deduced from the good relationships observed with the water-soluble fraction, that chromate is readily absorbed by the respiratory tract. Secondly, since ΔCr yielded the best correlations with ambient Cr(VI), a rapid response of the worker to his environment must have occurred (half-time in terms of hours). This is in agreement with the accumulation and release kinetics of the "uptake" compartments depicted in Fig. 3.6. Mathematical modelling determined that elimination half-times of 15 - 41 h (N=4) occurred for manual stainless steel welders in Tola *et al.*'s study (Tossavainen *et al.*, 1980), and 5 - 35 h (N=12) for those studied by Welinder *et al.* (1983). Thirdly, the non-zero y-intercept value for plots of urinary Cr *versus* soluble ambient Cr implies the existence of a compartment that decays with a longer half-time (see item D in Table 3.4). Welinder *et al.* (1983) assigned half-times of 14 d to infinity for the slowly decaying stores in welders, and estimated that on average the fraction of chromium originating from the slow compartment was 31% (range 11 - 82; N=12). Again, this observation is in agreement with the model discussed in Sections 3.4.2 and 3.4.3. And finally, the strongest correlations were observed when creatinine-corrected urinary chromium values were employed, relative to specific gravity adjusted and neat concentrations. In Section 3.4.3, we interpreted this result as evidence that the dependence of chromium excretion on urine flow is similar to that known for creatinine (see Section 3.5.5.2 for additional explanation).

A preliminary estimate of the scaling factor relating urinary chromium concentrations to ambient water-soluble Cr(VI) concentrations is obtained from the $\Delta$Cr increment data in Table 3.4 (item A). Although additional confirmation is required, this evaluation permits the prediction of the daily increase in urinary chromium levels equivalent to a range of water-soluble ambient Cr(VI) values. Results for some ambient concentrations are given in Table 3.5, including the TWAs recommended by ACGIH and NIOSH. Both of these latter ambient concentrations would result in mild elevation of chromium excretion.

The data in Tables 3.4 & 3.6 suggest that the relationships found between ambient chromium and urinary levels seem to hold for chromeplating workers as well. This group is also exposed to water-soluble chromates. The reports by Guillemin and Berode (1978), Bovet et al. (1977), and Franchini et al. (1983) indicate that urinary chromium concentrations during the mid-seventies in chromeplating shops were similar in magnitude to those reported in Figs. 3.9 and 3.11 for welders. Current exposure appears to have been reduced considerably by improved industrial hygiene measures (Franchini et al., 1983).

Tandon et al. (1977) have reported extremely high urinary chromium levels for electroplaters and paint-pigment workers (up to 1000 μg $l^{-1}$). Judging by the high value of 25 μg $l^{-1}$ found for control subjects, the analytical methods employed appear suspect, especially since no information about them is provided. As explained in footnote d to Table 3.6, the recent report by Lindberg and Vesterberg (1983) for electroplaters also seems to be fraught with technical error.

### 3.5.4 Chromium in Human Tissues and Hair

As pointed out in Section 3.5.1, the reference values for chromium in tissues of normal individuals are very dubious because of contamination during collection, handling and analysis. Unfortunately, authors reporting tissue chromium levels in exposed individuals fail to describe the analytical details of the methods employed, nor is the care exercised to prevent contamination outlined. Consequently, only those data that show a substantial increase over control values (>10-fold excess) can be considered reliable. A summary of the available data is provided in Table 3.7. It is clear that the lung is the main target organ. This is not surprising since direct deposition occurs there. The occurrence of large amounts of chromium in lymph tissue also occurs regularly. Accumulations in some of the other tissues may have occurred, but increases are not large enough relative to controls to conclude with confidence that this is so (see earlier comment).

The data in Table 3.7 unequivocally establishes the presence of high chromium levels in the lung tissues of both chromate manufacture workers and chrome platers, most of whom died of lung cancer. These pools appear to increase with the duration of exposure (Tsuneta *et al*., 1980; Tsuneta, 1982), and decay at a very slow rate ($t_{1/2}$ in y), since elevated levels still occurred 16 y (Mancuso, 1975) and 30 y (Teraoka, 1981) after last exposure. This finding is in agreement with the non-zero y-intercept values observed for plots of urinary chromium versus water-soluble chromium for welders (see Section 3.5.3), which implied such slowly decaying compartments.

Hair chromium levels are generally between 0.10 and 1.0 $\mu g\ g^{-1}$ (Parkhurst and Pate, 1979; Guthrie, 1982). Apparently, the significance of chromium concentrations in hair as an indicator of body stores is uncertain, although there is some evidence that diabetics have reduced amounts in hair. Indeed, elevated serum glucose levels have been correlated with increases in chromium excretion (Anderson et al., 1982a,b). Chromium in hair has not been employed extensively as an index to exposure (Jenkins, 1979). For tannery workers, Saner et al (1984) have reported chromium levels of 17 ± 4 $\mu g\ g^{-1}$ in hair and of 7 ± 1 $\mu g\ l^{-1}$ in urine (compared to 0.6 ± 0.1 $\mu g\ g^{-1}$ and 0.22 ± 0.03 $\mu g\ l^{-1}$ for unexposed controls).

## 3.5.5 Urinary Chromium as an Exposure Index: Recommendations and Justification

### 3.5.5.1 Short-Term Versus Long-Term Excretion

We conclude that urinary chromium is a suitable index of exposure to water-soluble chromates (i.e., Cr(VI)). The incremental increase in urine chromium during the working day ($\Delta$Cr) appears to be proportional to the ambient Cr(VI) level (Fig. 3.10, Table 3.4). Strong evidence exists that *in vivo* Cr(VI) is reduced to Cr(III). With reference to the model in Fig. 3.6, release of Cr(III) from the "uptake" compartments is characterized by a half-time of 2.2 d or less, and this is in agreement with the value of 1 - 2 d established for the elimination half-time of chromium excretion in welders exposed to Cr(VI) (Tossavainen et al., 1980). This suggests that the Cr(VI)-reduction hypothesis in man is correct. Because the "storage" compartments in Fig. 3.6 release Cr(III) slowly, and because slowly decaying pools have been identified in the respiratory tract of chromium workers, a residual chromium excretion in urine should persist after the initial decay of newly absorbed chromium. As already indicated, the non-zero intercepts in plots such as in Figs. 3.9 and 3.11 may be taken as evidence of such residual (base) release in welders.

On the basis of the necropsy data reviewed in Table 3.7, one might suspect that the deposits in the respiratory tract of chromium workers are significant and, together with the naturally slow-releasing "storage" compartments, might therefore be expected to determine the long-term decay kinetics. As already indicated, the size of such pools appears to increase with the duration of exposure (Tsuneta et al., 1980; Tsuneta, 1982). Furthermore, the decay half-time may be expected to depend strongly on the oxidation state and solubility in biological fluids of the deposits, and other properties of solids such as crystallinity, surface morphology and surface reactivity (e.g., adsorption of protein and cell lysis). There is some experimental evidence for this conclusion. Bragt and van Dura (1983) administered 3 chromate salts by intratracheal injection to rats. They estimated that the release into the blood was 17 µg Cr $l^{-1}d^{-1}$ for sodium chromate (water soluble, see Table 3.2), 11 µg Cr $l^{-1}d^{-1}$ for zinc chromate (water insoluble) and 0.2 µg Cr $l^{-1}d^{-1}$ for lead chromate (water insoluble). (The zinc and lead salts were prepared by precipitation and most likely were amorphous rather than crystalline; the injected material had maximal diameters of 2.0 ± 0.9 µm and 3 ± 2 µm, respectively.) All three compounds were removed from the lung with half-times ($t_{1/2}$) of around 2 d, and from the whole body with $t_{1/2}$ of 2 - 5 d (fast initial phase in first 4 days) and $t_{1/2}$ = 31 - 95 d (slow subsequent phase). After the initial fluxes following the administration of the dose had settled down, all three chromates were lost from the blood and excreted into the urine with $t_{1/2}$ = 2 - 6 d. Loss in the faeces was monophasic ($t_{1/2} \sim 1$ d). At 10 days after the injection, the amount of the chromium dose excreted in the faeces varied from 75% (Pb), to 45% (Zn) and 21% (Na), compared to total excretion in the urine of 20% (Na and Zn) and 2% (Pb). On day 51, the residual chromium content of the body was 8.3% of the initial sodium chromate dose, while it was 5.3% and 12.5% for the zinc and lead compounds, respectively. Langård *et al*. (1978) have also found in inhalation studies with rats that zinc chromate was absorbed quickly and excreted slowly. From these animal studies one concludes that water solubility is not a good index to the rate of internal mobilization and pathways of metal chromates. The similarity for all three chromates in the half-time values for whole-body chromium elimination, for the decay of blood chromium levels, and for the urinary excretion rates is strongly suggestive that absorbed chromate is handled in the same manner in the rat regardless of the source. The low urinary and high fecal excretion in the case of lead chromate most likely reflects a different clearance mechanism. Interestingly, retention of stainless steel manual-welding fumes in the lungs of man and rat have been shown to be significantly higher, and clearance lower, than of mild steel manual-welding fumes (Kalliomäki _et al_., 1983a). For nickel workers the scaling factor that relates urinary nickel (in $\mu g\ l^{-1}$) to ambient nickel levels (in $mg\ m^{-3}$) had values of 700 for exposures to aerosols of dissolved nickel salts encountered in electrolytic refining and 20 - 85 for exposures to particulate nickel compounds encountered in pyrometallurgical refining (Nieboer _et al_., 1983a). The magnitude of such linear scaling factors is therefore diagnostic of a particular working environment, and likely reflects the speciation, composition, surface morphology and reactivity, solubility and particle size of the ambient particulates.

Cr(III) compounds appear generally to be taken up less readily than Cr(VI) compounds (Section 3.4.1). Like welders (Tola _et al_., 1977; Stern, 1982) and ferrochromium workers (Langård _et al_., 1980), chromate manufacturing workers (Bourne and Yee, 1950; Buckell and Harvey, 1951; Gafafer, 1953) are known to be exposed to chromium in more than one oxidation state, namely Cr(III) and Cr(VI) compounds. In addition, welders and ferrochromium workers are also known to be exposed to particulates of chromium metal or its alloys. Not surprisingly therefore, Kalliomäki et al. (1981) found a good correlation ($r=0.85$) between urinary chromium levels and the cumulative exposure (exposure time in years x % of time performing manual metal arc stainless steel welding), as well as between the average measured remanent magnetic fields of the chest and the cumulative exposure ($r=0.82$). (The remanent magnetic field of the chest area is a direct measure of the metal contaminants that have accumulated in the lungs.) In follow-up studies, other correlations have been reported. Among manual stainless steel welders, the retention rate of magnetic dust in the lungs (the remanent magnetic field divided by exposure period in years) showed a relationship ($P < 0.01$) with ambient chromium levels [total Cr or Cr(VI)]. A strong correlation with the daily mean increase in whole blood chromium also existed (Rahkonen et al. 1983). For manual mild-steel welders, Kalliomäki et al. (1983b) estimated that about 1% of the dust deposited in the lung was retained (~70 mg $y^{-1}$ at current exposures), and that the average clearance rate of retained material was 20% per year ($t_{1/2} = 3.5$ y). This long half-time corresponds to a biological residence time of 5 y, and confirms the existence of long-term deposits.

To reiterate, the daily incremental increase in urine chromium (ΔCr) appears to be a better index of current exposure to water-soluble Cr(VI) than the end-of-shift value, because of the large contribution of long-term excretion. This conclusion follows clearly from the analysis by Sjögren _et al_. (1983), as well as from the data in Table 3.4, although it was not realized by these researchers. A small contribution of water-soluble Cr(III) to ΔCr might also occur, although such compounds have low abundance in most industrial airborne particulates (Gafafer, 1983; Stern, 1982) and might be expected to be absorbed less readily. Consequently, the water-soluble ambient chromium concentration is a good measure of the short-term release of chromium in urine. Water extraction of air samples from locations in chromate production plants (Gafafer, 1953) and from airborne welding fumes (Thomsen and Stern, 1979; Stern 1982) yield only Cr(VI). Mild alkali elution (with 1% carbonate) appears to dissolve the same fraction, while leaching with hot NaOH-$Na_2CO_3$ is effective in removing water-insoluble chromate constituents as well (total Cr(VI)) (Thomsen and Stern, 1979; Bhargava _et al_; 1983; Moreton _et al_., 1983). In view of the animal work by Bragt and van Dura (1983) reviewed in detail above, the measurement of _total Cr(VI)_ seems inherently of greater biological significence than the evaluation of _water-soluble Cr(VI)_. Supplementary to _total Cr(VI)_ levels, the _total ambient chromium level_ should be an index to the contaminant pool building up in the body, and thus be determinative in the long-term basal urinary excretion. As suggested above, the naturally slow-releasing "storage" compartments will necessarily contribute to this residual excretion. The *in vivo* mobilization of chromium deposits of exogenous origin may be expected to depend on the composition of the material to which individuals were exposed, and thus the work category, as already discussed. End-of-shift urinary chromium reference values (sum of short- and long-term excretion) of 20 - 30 $\mu g\ l^{-1}$ are recommended in some countries (see Tola *et al.*, 1977; Kalliomäki *et al.*, 1981; Sjögren *et al.*, 1983). Of course, as pointed out by Rantanen *et al.* (1982), action levels for biological monitoring are arbitrary and serve only as an adjunct to environmental monitoring.

### 3.5.5.2 Creatinine Adjustment of Urinary Chromium

In Section 3.4.3, the evidence was reviewed which suggests that Cr(III) is largely excreted by glomerular filtration without significant tubular reabsorption. If confirmed, this indicates that creatinine adjustments do correct appropriately for differences in urine flow between individuals. On the other hand, if tubular reabsorption were to occur to a significant degree, as appears to be the case for Ni(II) and solutes (Nieboer *et al.*, 1983a; Sanford *et al.*, 1984), then creatinine-adjustments would overcompensate for dilution.

For a single person, Araki (1980) has shown that the relationship in Eqn (3.6) has general application for a number of analytes, including lead, creatinine, and total solutes.

$$U_i V^{b_i} = U_i^o \tag{3.6}$$

In this Eqn, $U_i$ is the concentration of "i" in the urine, V is the urine flow (ml $min^{-1}$ or l $d^{-1}$), $U_i^o$ is the urinary concentration of "i" at an excretion rate of 1 ml $min^{-1}$ or 1 l $d^{-1}$ (and is arbitrarily designated the standard analyte excretion rate), and $b_i$ is the volume exponent or power coefficient. For creatinine-like substances which are largely excreted by glomerular filtration, $b_i = 1.0$; while for substances that are subject to tubular reabsorption $b_i < 1.0$, such as appears to be the case for lead ($b_{Pb} = 0.75$), solutes ($b_{solute} = 0.67$) and also nickel ($b_{Ni} = 0.67$) (Araki, 1980; Sanford *et al*., 1984). When $b_i = 1.0$, the excretion rate of an analyte (amount per unit time) is independent of urine flow and simple dilution effects regulate its concentration. In instances when $b_i \neq 1.0$, the analyte excretion rate (i.e., UV; wt per unit time) has an implied dependence on urine flow.

Eqn (3.7) may be derived from Eqn (3.6), providing $b_{creat} = b_{Cr} = 1.0$.

$$U_{Cr} = \frac{U_{Cr}^o}{U_{creat}^o} U_{creat} \tag{3.7}$$

Since $U^{o}_{Cr}$ and $U^{o}_{creat}$ are constants, a linear relationship might be expected between $U_{Cr}$ and $U_{creat}$, providing substances are excreted at a constant rate (amount per unit time). The latter condition is approximated by creatinine, and may only be expected for chromium for healthy individuals that are not occupationally exposed and are on a normal diet. Since such relationships are observed (Section 3.4.3; Saner, 1980; Anderson et al., 1983), their existence may be correctly interpreted as indicating that chromium is excreted in a creatinine-like manner (i.e., by glomerular filtration). Creatinine-correction of urinary chromium therefore appears justified.

Recent data for Ontario nickel workers suggest that the specific gravity of urine specimens at a volume flow rate of 1 ml $min^{-1}$ was 1.021 ± 0.001 (Sanford et al., 1984). Since $b_{solute}$ = 0.67 (see Eqn 3.6), specimens with flow rates in the range 1.00 ± 0.25 ml $min^{-1}$ have specific gravities between 1.018 and 1.025. (Specific gravity - 1.000 may be taken as a measure of the solute concentration.) Rejection of specimens with specific gravities values outside this range would assure that dilution effects are minimal. A detailed analysis of the feasibility and usefulness of biological monitoring in the nickel producing industry suggests that group averages also permit valid comparisons of urinary nickel values, most likely because the mean urine flow rates for groups of workers are similar (Nieboer et al., 1983a).

### 3.5.5.3 Proposed Relationship Between Chromium in Plasma and Urine

When it is assumed that chromium is excreted primarily by glomerular filtration, it is possible to predict a relationship between plasma (serum), $P_{Cr}$, and urine chromium ($U_{Cr}$) concentrations. Eqn (3.8) follows directly from the conventional clearance formula after correction for the fraction of chromium ($\alpha_{Cr}$) not bound to protein (i.e., that available for filtration). Because chromium is an essential metal, reabsorption might be postulated for very low plasma concentrations, and this explains the intercept b in Eqn (3.8).

$$P_{Cr} = \frac{V}{\alpha_{Cr} C_{Cr}} U_{Cr} + b \qquad (3.8)$$

$C_{Cr}$ is the clearance of chromium (volume of plasma cleared per minute; units of ml min$^{-1}$); and all concentrations are in $\mu$g l$^{-1}$. In a group of individuals, such as workers, V averages near 1.0 ml min$^{-1}$ (Nieboer _et al_., 1983a); $\alpha_{Cr}$ is known to be 0.055 (Lim _et al_., 1983); and $C_{Cr}$ would have the value 117±20 ml min$^{-1}$ if glomerular filtration dominates, and if normal kidney performance exists (Faulkner and King, 1976; Nieboer _et al_., 1983a). Plugging these values into Eqn (3.8), and considering the consensus normal value of $P_{Cr}$ as 0.15 $\mu$g l$^{-1}$ and of $U_{Cr}$ as 0.6 $\mu$g l$^{-1}$, then Eqn (3.9a) follows.

$$P_{Cr} = 0.15\ U_{Cr} + 0.06 \quad \text{(neat urine)} \qquad (3.9a)$$

$$P_{Cr} = 0.25\ U_{Cr} + 0.06 \qquad (3.9b)$$

(creatinine-adjusted urine, $\mu g\ Cr\ g^{-1}$)

The slope in Eqn (3.9b) was estimated by multiplying that in Eqn (3.9a) by the urine/ambient chromium scaling factor ratio for neat and creatinine-corrected urines (see Table 3.4, entry A). The recent data reported by Rahkonen et al. (1983) for welders fit Eqn (3.9b) extremely well. Eqn (3.9a) implies that for $U_{Cr} > 5\ \mu g\ l^{-1}$, $P_{Cr}$ is about 15% of the value of $U_{Cr}$. From the analytical point of view, this makes the analysis of urine more attractive than plasma, as is readily illustrated by the following examples. The reference $U_{Cr}$ value mentioned earlier of 30 $\mu g\ l^{-1}$ corresponds to 4.6 $\mu g\ l^{-1}$ of chromium in plasma; while a value of $U_{Cr} = 100\ \mu g\ l^{-1}$ (see Figs. 3.9 and 3.11) yields $P_{Cr} = 15\ \mu g\ l^{-1}$. Additional measurements are required to confirm the relationships in Eqns (3.9a and 3.9b).

And finally, since absorption of chromate results in the accumulation of chromium in erythrocytes and absorption of Cr(III) compounds does not (Section 3.4.2), erythrocyte chromium concentration (or perhaps whole blood chromium) would appear to be a specific index of exposure to Cr(VI). Indeed, the daily mean increase in whole blood chromium has been strongly correlated with ambient Cr(VI) for manual stainless steel welders (Rahkonen et al., 1983). Plasma chromium concentrations were about 60% of whole blood values, indicating a preference for erythrocytes.

## 3.6 Models of Toxicity

### 3.6.1 Introductory Remarks

An understanding of the models described for the mutagenicity/carcinogenicity and contact sensitivity associated with chromium compounds requires that the reader be familiar with the material on the chemical and biochemical reactivity of chromium outlined in previous sections of this document. Specific cross-references are not provided.

### 3.6.2 Chromium Mutagenicity and Carcinogenicity

The ability of the chromate anion to diffuse readily through cell membranes and its reasonable longevity at physiological pH, even though it is relatively strongly oxidizing, are crucial to the toxicity model depicted in Figs. 3.12 and 3.13. Chromate is not only reduced to Cr(III) directly by extracellular and intracellular low-molecular-weight molecules (e.g., glutathione, ascorbic acid, lactic acid) and proteins, but also enzymatically by cellular microsomes. Furthermore, because of its avid non-labile complex-formation ability, Cr(III) accumulates in storage compartments (such as cell nuclei) from which it is released slowly. Providing Cr(III) is in the correct ligand environment, it too can be transported across biological membranes; both by diffusion as low-molecular-weight complexes (e.g., of amino acids, and oxalate), and when bound to protein by endocytosis (e.g., transferrin). All these transport and distribution mechanisms are depicted in Figs. 3.12 and 3.13.

Inside the nucleus, Cr(III) is believed to be the active form. Because the Cr(III) cation interacts strongly with nuclear enzymes, proteins, nucleotides and polynucleotides, Cr(III)-induced lesions may result in mutations (Loeb *et al*., 1974; Loeb and Zakour, 1980). For example, Cr(III) has been demonstrated *in vitro* not only to inhibit DNA synthesis, but also to increase infidelity (Sirover and Loeb, 1976). In the current somatic model of cancer, the unified concept of carcinogenesis holds that carcinogenic potential exists in all cells, and that "neoplastic transformation involves mutation or translocation of a cellular oncogene or of a nearby regulatory gene that normally represses the oncogenes" (Sunderman, 1984).

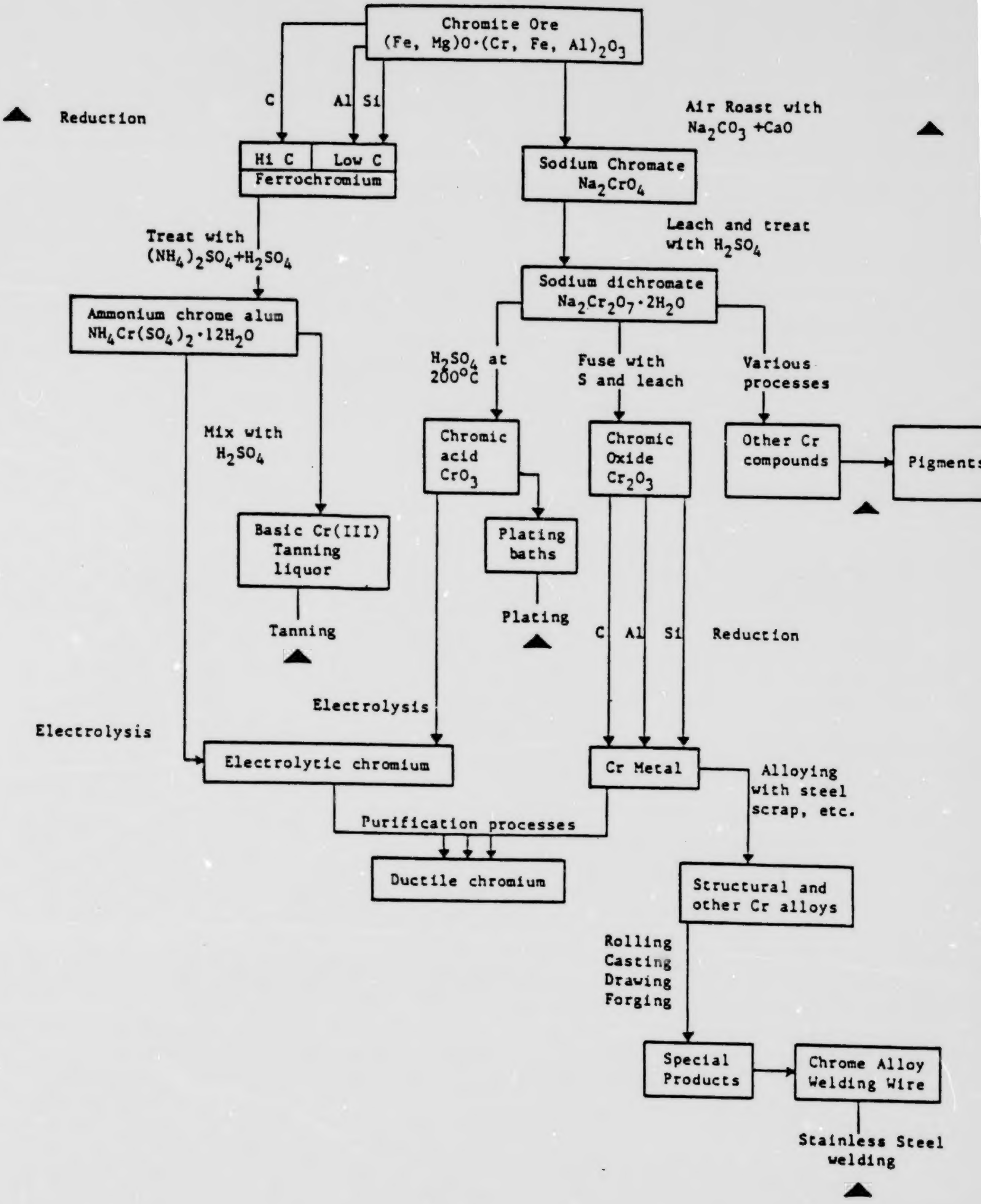
Recently, Hansen and Stern (1983) concluded that intracellular bioavailability is an important determinant in mutagenicity. They found for studies with nickel compounds that equal transformation rates of baby hamster kidney cells (BHK-21) occurred at equal toxicities, regardless of the source of nickel. Both soluble and insoluble nickel compounds were studied. Equal toxicity, as measured by survival rates, was interpreted to correspond to equal intracellular concentrations of $Ni^{2+}$. Consequently, nickel compounds which are taken up as particulates by phagocytosis, and which have favourable dissolution rates, may be expected to be most effective. This was found to be the case, since solid nickel subsulfide was most potent. Presumably, intracellular bioavailability assures that the carcinogenic form of metals can accumulate in the nucleus rather than undergoing extracellular transport. Since pools of nickel compounds have been confirmed in target tissues (nasal mucosa and lung) of nickel production workers afflicted with cancer (Nieboer *et al*., 1983a), as well as chromium accumulation in the lung tissue of chromate workers with respiratory cancer (see Table 3.7), bioavailability appears assured. Animal studies indicate (see Chapter 4) that the relatively insoluble chromates are the most effective carcinogens. Relatively insoluble nickel compounds are also the most potent, of which nickel subsulfide is the prime example. Perhaps soluble chromates and nickel compounds do not accumulate intracellularly as effectively, since they are characterized by relatively short biological half-lives. A substantial fraction of that absorbed is excreted shortly after exposure, no doubt because the Ni(II) ion and the chromate anion (see Fig. 3.12) can be and are removed from cells. On this account, soluble forms of carcinogenic metals may not be able to exercise their full carcinogenic potential.

### 3.6.3 Molecular and Cellular Basis of Chromium Contact Dermatitis

As in mutagenesis and carcinogenesis, Cr(III) appears to be the active form in the pathogenesis of chromium contact dermatitis. After penetrating the skin, perhaps as the chromate anion, Cr(III) is visualized as interacting with skin proteins (see Fig. 3.14). The Cr(III)-protein adduct is the form that is recognized by antigen-inexperienced T-cells (see Fig. 3.15), and it is postulated that dendritic cells in the epidermis, called Langerhans' cells, participate in the formation of the antigenic complex. These macrophage-like cells are not only located in the skin, but also are found in the draining lymph node. It has been suggested that antigenic recognition might occur in both places, and that Langerhans' cells mediate both the induction and elicitation of contact sensitivity (Polak, 1983). Additional detail is provided in Fig. 3.15.

Figure 3.1 A simplified flow chart summarizing the steps in the production of metallic chromium and some of the important commercial chromium compounds. Processes for which occupational exposure data are available are denoted by the symbol ▲. Reproduced with permission from Stern (1982).

## PRODUCTION OF CHROMIUM METAL ALLOYS AND COMPOUNDS FROM CHROMITE ORE


Chromite Ore
(Fe, Mg)O·(Cr, Fe, Al)2O3
C
Al
Si
Reduction
Air Roast with
Na2CO3 +CaO
Hi C
Low C
Ferrochromium
Sodium Chromate
Na2CrO4
Leach and treat
with H2SO4
Treat with
(NH4)2SO4+H2SO4
Sodium dichromate
Na2Cr2O7·2H2O
Ammonium chrome alum
NH4Cr(SO4)2·12H2O
H2SO4 at
200°C
Fuse with
S and leach
Various
processes
Mix with
H2SO4
Chromic
acid
CrO3
Chromic
Oxide
Cr2O3
Other Cr
compounds
Pigments
Basic Cr(III)
Tanning
liquor
Plating
baths
Tanning
Plating
C
Al
Si
Reduction
Electrolysis
Electrolysis
Electrolytic chromium
Cr Metal
Alloying
with steel
scrap, etc.
Purification processes
Ductile chromium
Structural and
other Cr alloys
Rolling
Casting
Drawing
Forging
Special
Products
Chrome Alloy
Welding Wire
Stainless Steel
welding

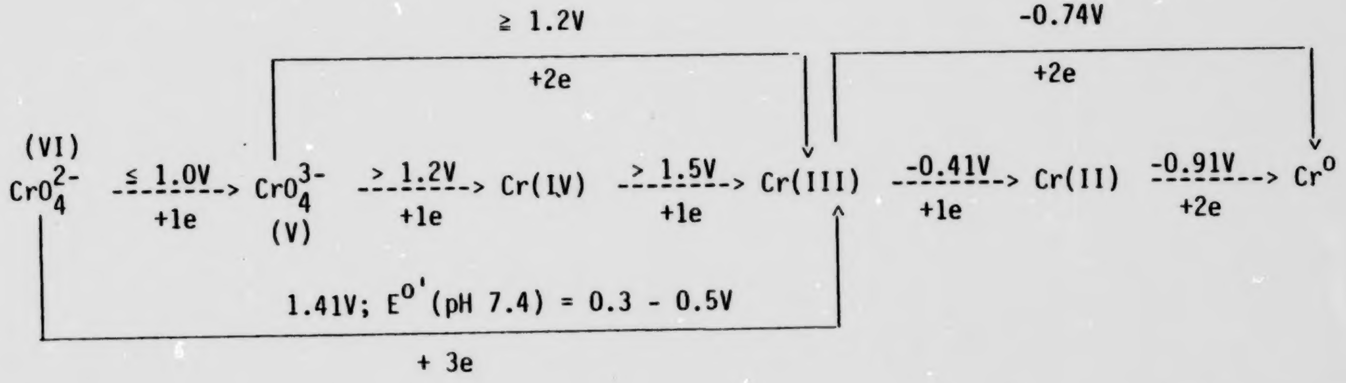
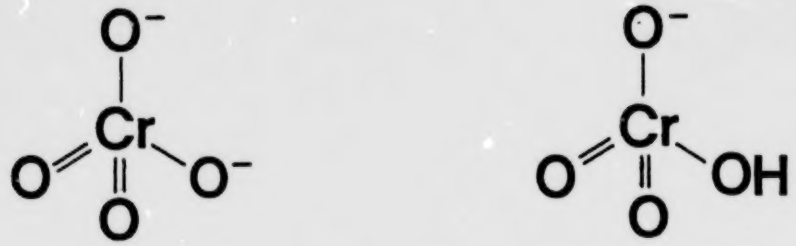

≥ 1.2V
+2e
-0.74V
+2e
(VI)
CrO$_4^{2-}$
≤ 1.0V
+1e
CrO$_4^{3-}$
(V)
> 1.2V
+1e
Cr(IV)
> 1.5V
+1e
Cr(III)
-0.41V
+1e
Cr(II)
-0.91V
+2e
Cr$^0$
1.41V; E$^{0'}$(pH 7.4) = 0.3 - 0.5V
+ 3e

Figure 3.2 Reduction-potential diagram for chromium. The standard electrode potential in volts (E°) is indicated above the arrow linking two species, and refers to rather concentrated solutions (standard state, of unit activity; $\geq$ 1 M); for more dilute solutions, the E° values are more negative. Of the two species linked by an arrow, the reduced form at the right of the arrow is favoured when E° is positive, because of energy considerations (see Table 3.1, footnote a, for additional explanation); conversely, that at its left is more stable when E° is negative. For $CrO_4^{2-}$ and $CrO_4^{3-}$, the E° value is pH dependent because protons are consumed in the reaction; as shown for $CrO_4^{2-}$ its value (given the symbol E°' for pH $\neq$ 0) is - 0.5V at pH 7.4 (see Table 3.1, footnote a, for supplemental rationale). Diagram was constructed from data reported in Westheimer (1949), Beattie and Haight (1972), and Cotton and Wilkinson (1980).



chromate

monohydrogenchromate

Figure 3.3 Structural formulae for the biologically important forms of Cr(VI) at the physiological pH of 7.4; $HCrO_4^-$ constitutes 3% of the total, while the remainder exists as $CrO_4^{2-}$.

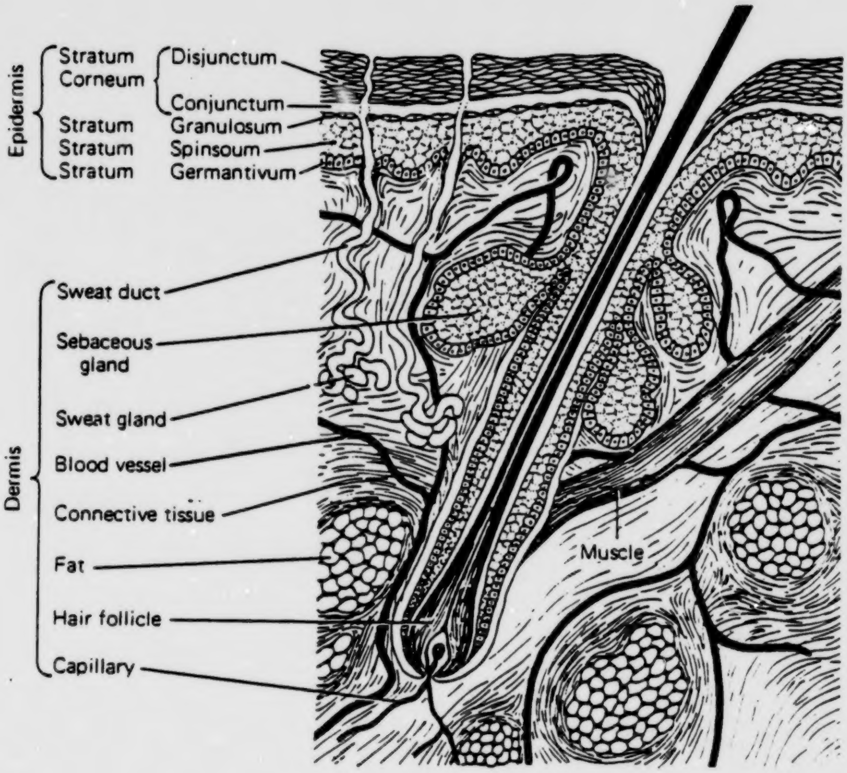

Epidermis
Stratum Corneum
Disjunctum
Conjunctum
Stratum Granulosum
Stratum Spinsoum
Stratum Germantivum
Dermis
Sweat duct
Sebaceous gland
Sweat gland
Blood vessel
Connective tissue
Fat
Hair follicle
Capillary
Muscle

Figure 3.4 Diagram of a cross-section of human skin. Reproduced with permissio from Klaassen, 1980.

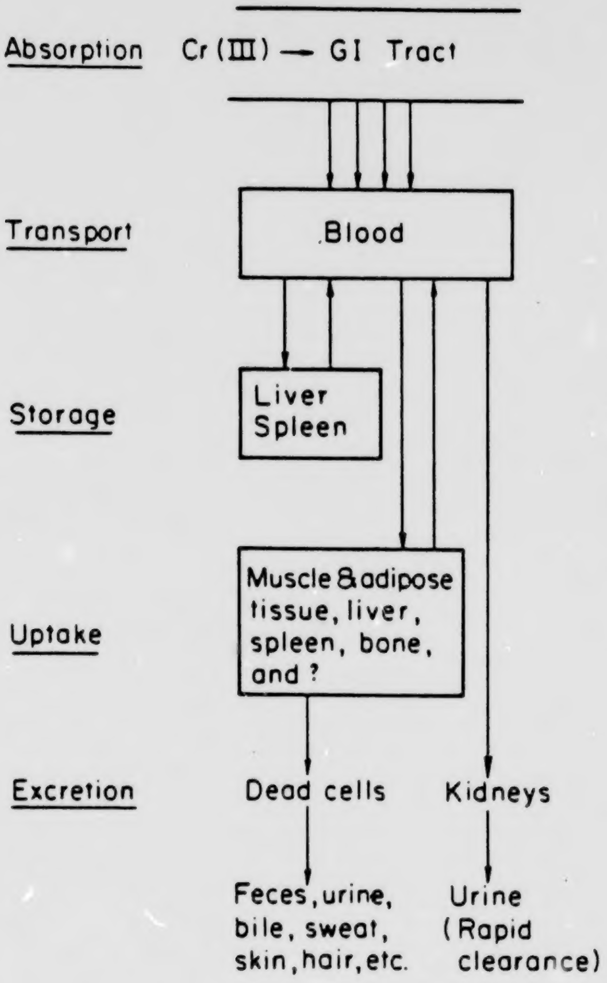

Absorption
Cr (III) — GI Tract
Transport
Blood
Storage
Liver
Spleen
Uptake
Muscle & adipose tissue, liver, spleen, bone, and ?
Excretion
Dead cells
Kidneys
Feces, urine, bile, sweat, skin, hair, etc.
Urine (Rapid clearance)

Figure 3.5 Proposed pathways for chromium (III) in the human body, based on human and animal data. Cr(III) is transported bound mainly to plasma transferrin. Reproduced with permission from Lim *et al*. (1983).

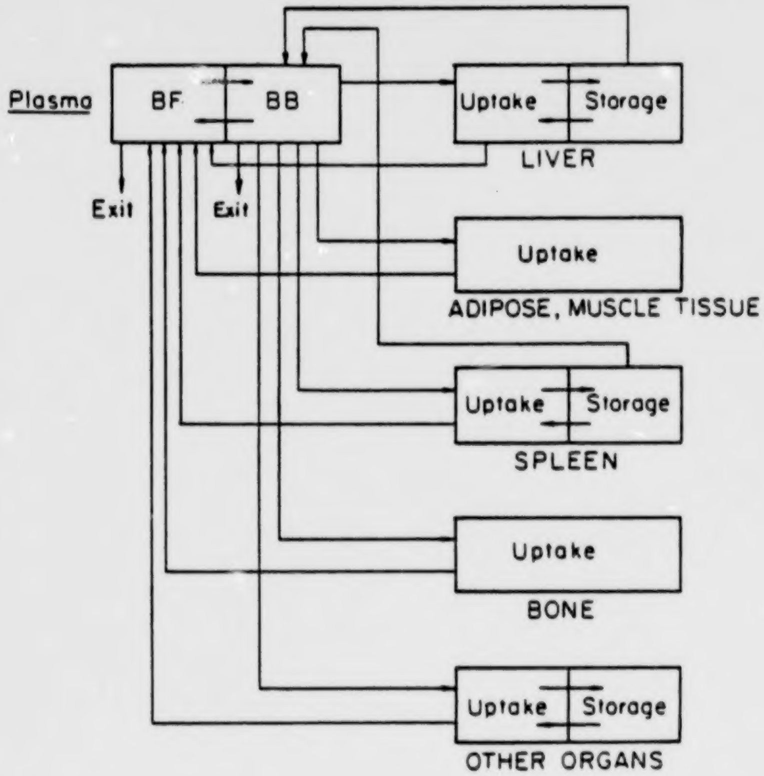

Plasma
BF
BB
Exit
Exit
Uptake
Storage
LIVER
Uptake
ADIPOSE, MUSCLE TISSUE
Uptake
Storage
SPLEEN
Uptake
BONE
Uptake
Storage
OTHER ORGANS

Figure 3.6 Multicompartment physiological model for chromium(III) kinetics in the human body. Plasma chromium is the central compartment, consisting of two subcompartments, the plasma protein-bound (BB; i.e., to transferrin) and the plasma protein-unbound (BF) chromium. Return from the organs is shown to occur in the unbound form, but may well also take place as BB. For a mathematical solution, the "uptake" compartments take up and release Cr(III) relatively rapidly (half-times, $t_{1/2}$, of $< 1$ h to 2 days); while the uptake in the "storag compartments occurs more slowly ($t_{1/2}$, 4d), with extremely slow release ($t_{1/2}$, 315d). Excretion occurs via kidney clearance ($t_{1/2}$, 3.5 h) and other slower routes (e.g., desquamated cells and faeces; $t_{1/2}$ = 7d). "Other organs" denote those that could not be resolved by the whole-body scintillation scanning technique, including kidney, heart and CNS. Reproduced with permission from Lim _et_ _al_. (1983).

Figure 3.7 Chronological decrease in "normal" chromium concentrations reported for sera (plasma) of unexposed individuals. Contamination during collection, handling and analysis accounts for both the drastic downward trend and the improvement in precision. The error bars denote the standard deviation, and the number of data points contributing to the mean are shown for each histogram. The consensus value is ~ 0.15 µg $\ell^{-1}$. Based on the compilation reported in Guthrie (1982).

CHROMIUM CONCENTRATIONS IN SERUM AND PLASMA

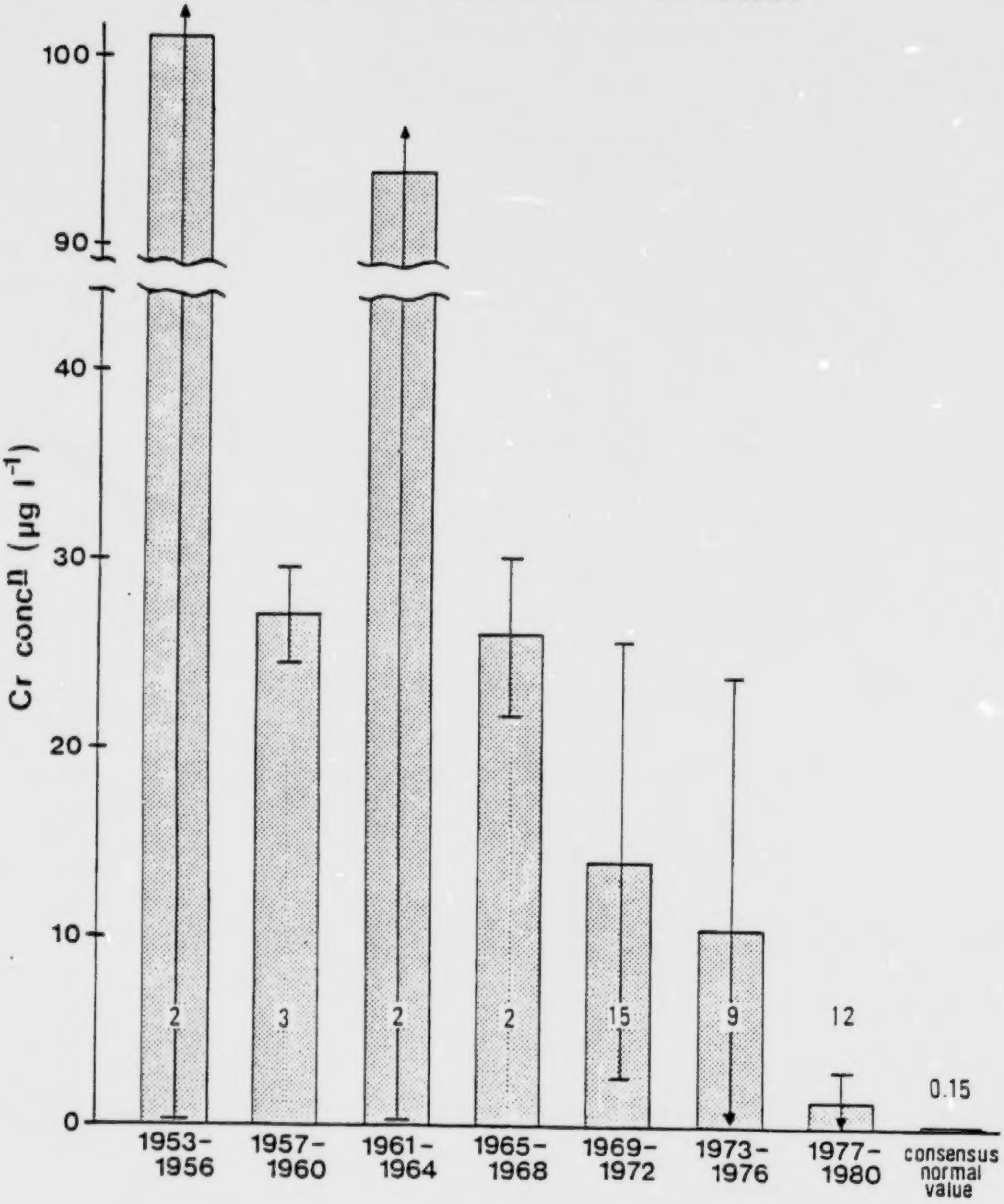
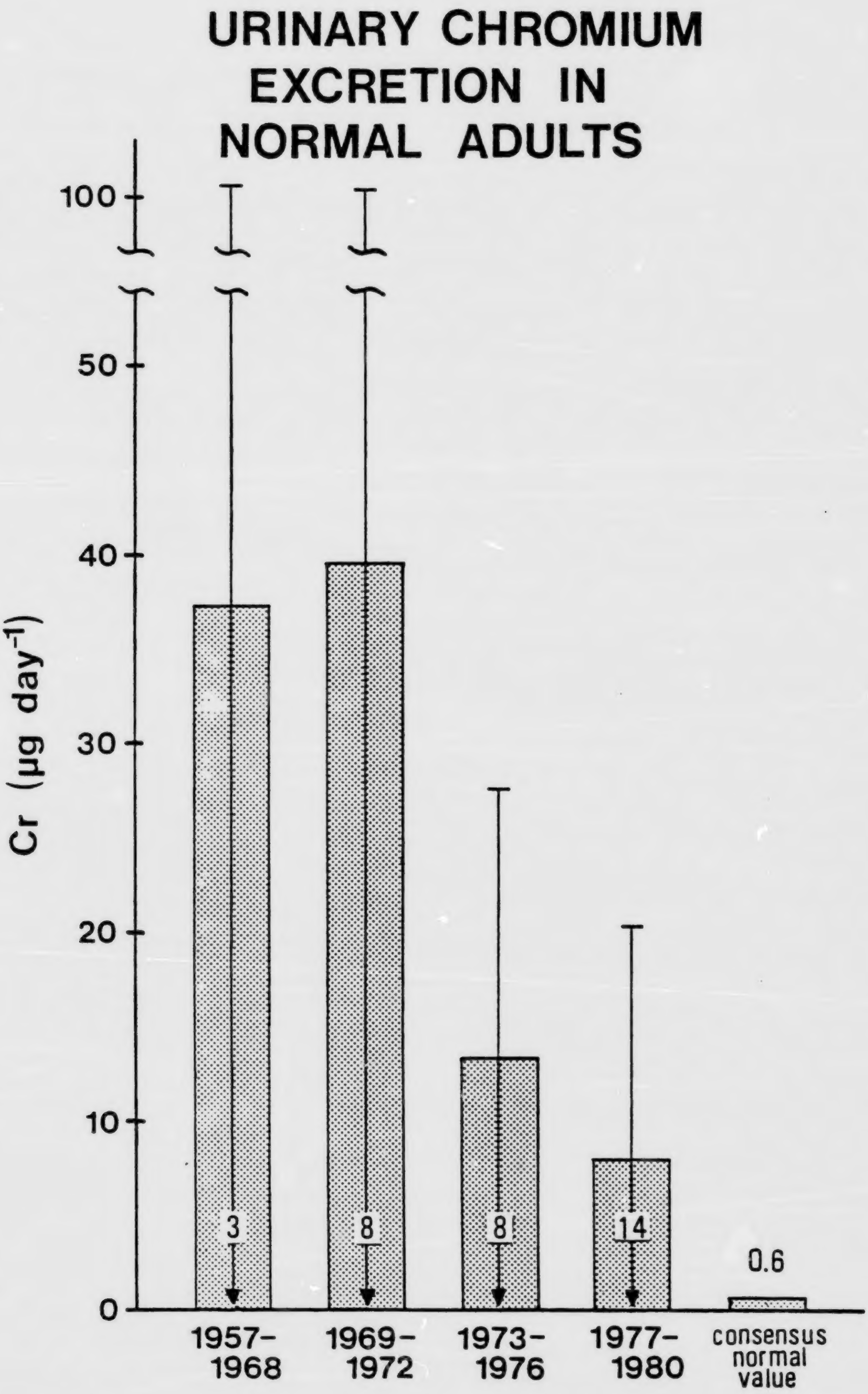


Figure 3.8 Chronological decrease in "normal" urinary chromium excretion in unexposed adults. Contamination during collection, handling and analysis adequately explains the drastic downward trend. The error bars denote the standard deviation, and the number of data points averaged are shown for each histogram. The consensus value is ~ 0.6 μg $d^{-1}$. Based on the compilation reported in Guthrie (1982).



URINARY CHROMIUM EXCRETION IN NORMAL ADULTS

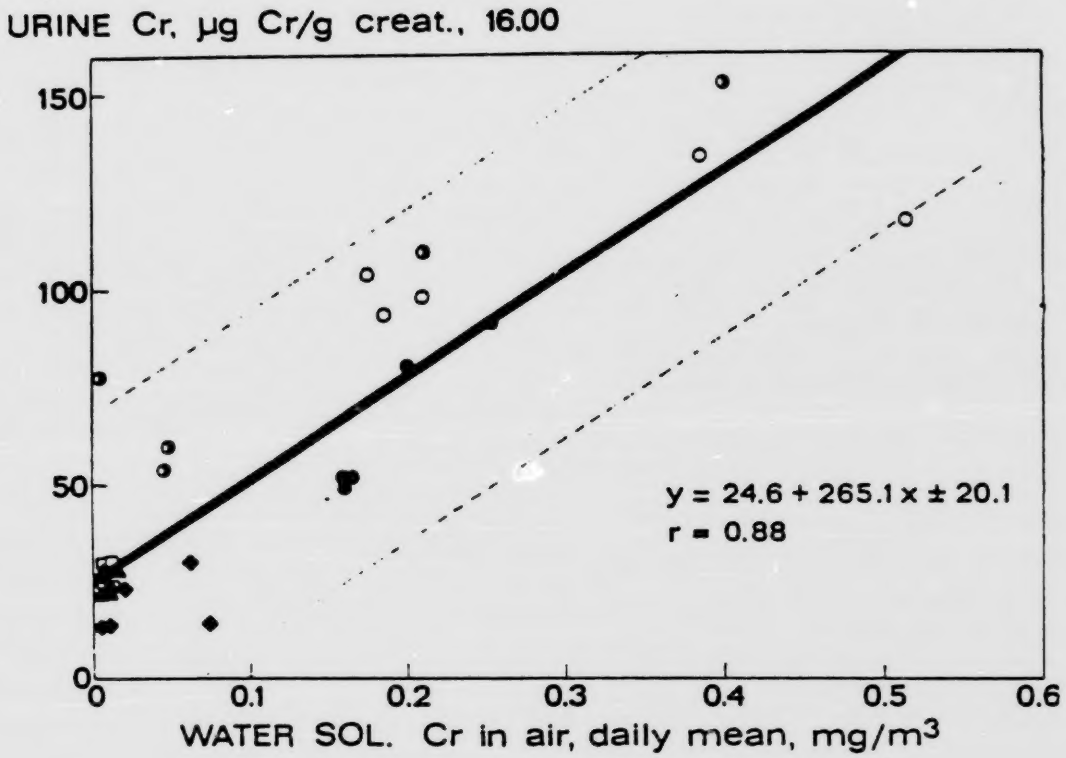


Figure 3.9 Relationship between the water-soluble chromium concentration (primarily Cr(VI)) in the air and the creatinine-corrected urinary chromium for welders. Urine specimens were collected at the end of the work shift (at 1600) after showering. Six welders were monitored daily for one week; four of them did manual stainless steel welding, another did inert gas stainless steel welding, and the sixth was only involved in the welding of mild steel. See Table 3.4 for complementary data on the cohort. Reproduced with permission from Tola et al. (1977).

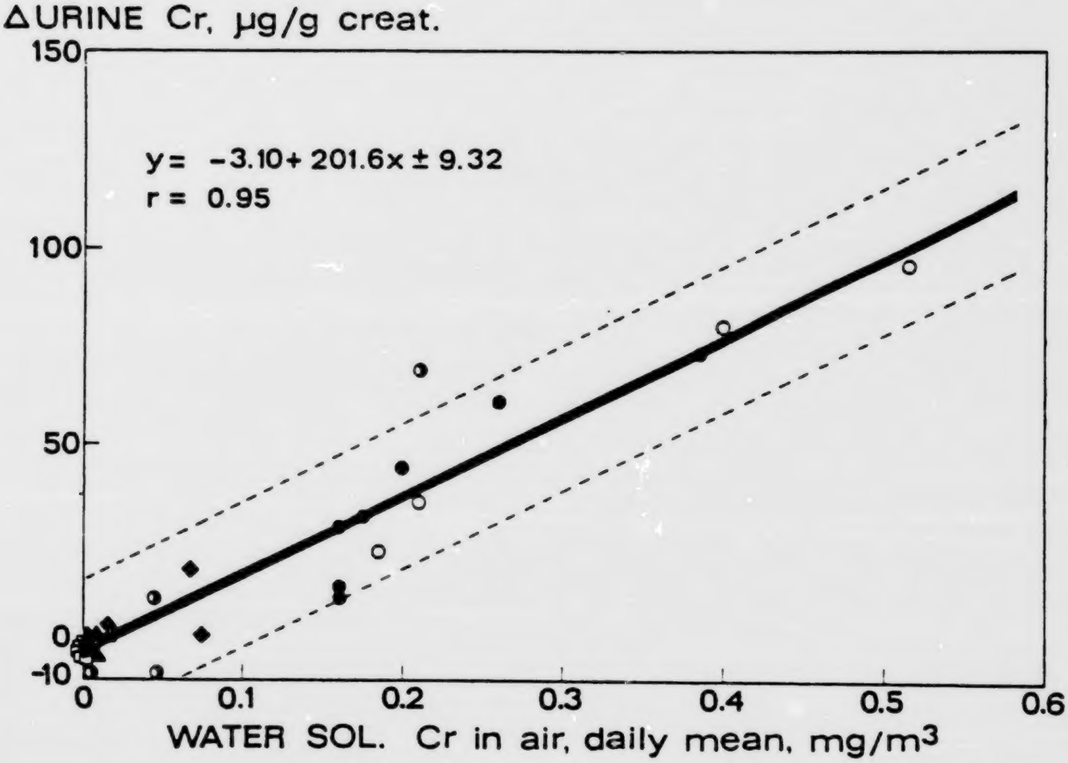


Figure 3.10 Relationship between the increase in creatinine-corrected urinary chromium concentration (ΔCr) during the workday for the group of welders referred to in Fig. 3.9. The preshift morning measurement (0700) was subtracted from that obtained for the afternoon collection (1600). Reproduced with permission from Tola et al. (1977).

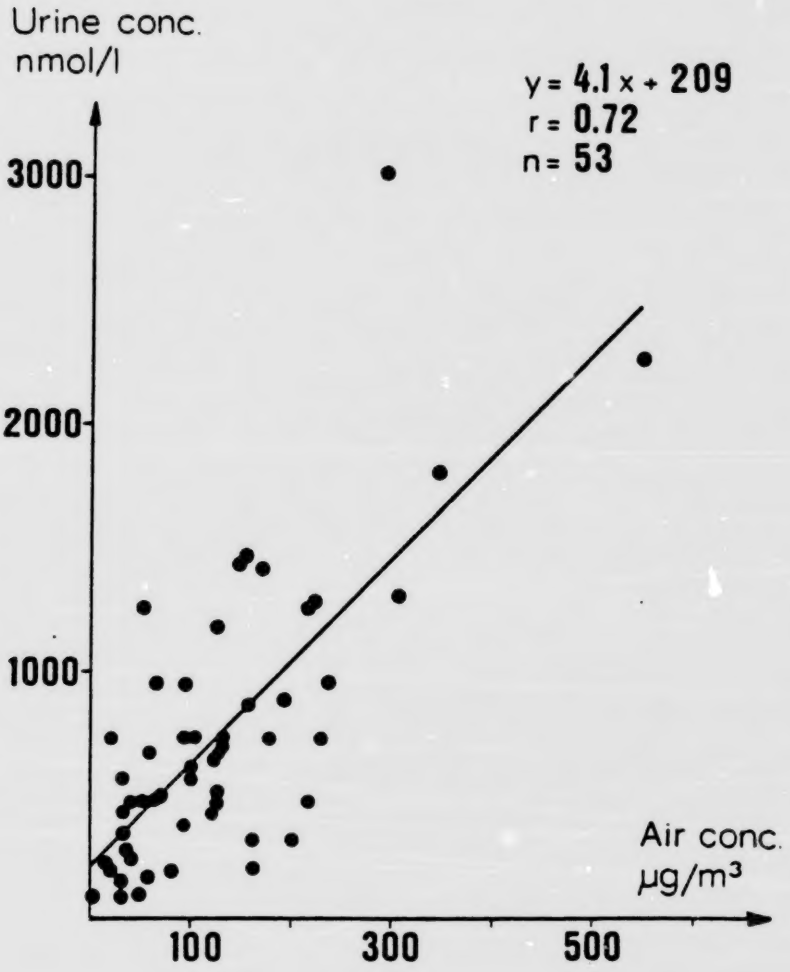


Figure 3.11 Eight-hour TWA air concentrations and unadjusted post-shift (end-of-day) urine concentrations of chromium for stainless steel welders. Reproduced with permission from Sjögren *et al.*, 1983. To express the chromium concentration in $\mu g\ \ell^{-1}$ multiply by 0.052.

Figure 3.12 Suggested model of Cr(VI) uptake in the lungs; M, mitochondria; N, nucleus; RBC, red blood cell; GSH, glutathione. Reproduced with permission from Langård (1982).

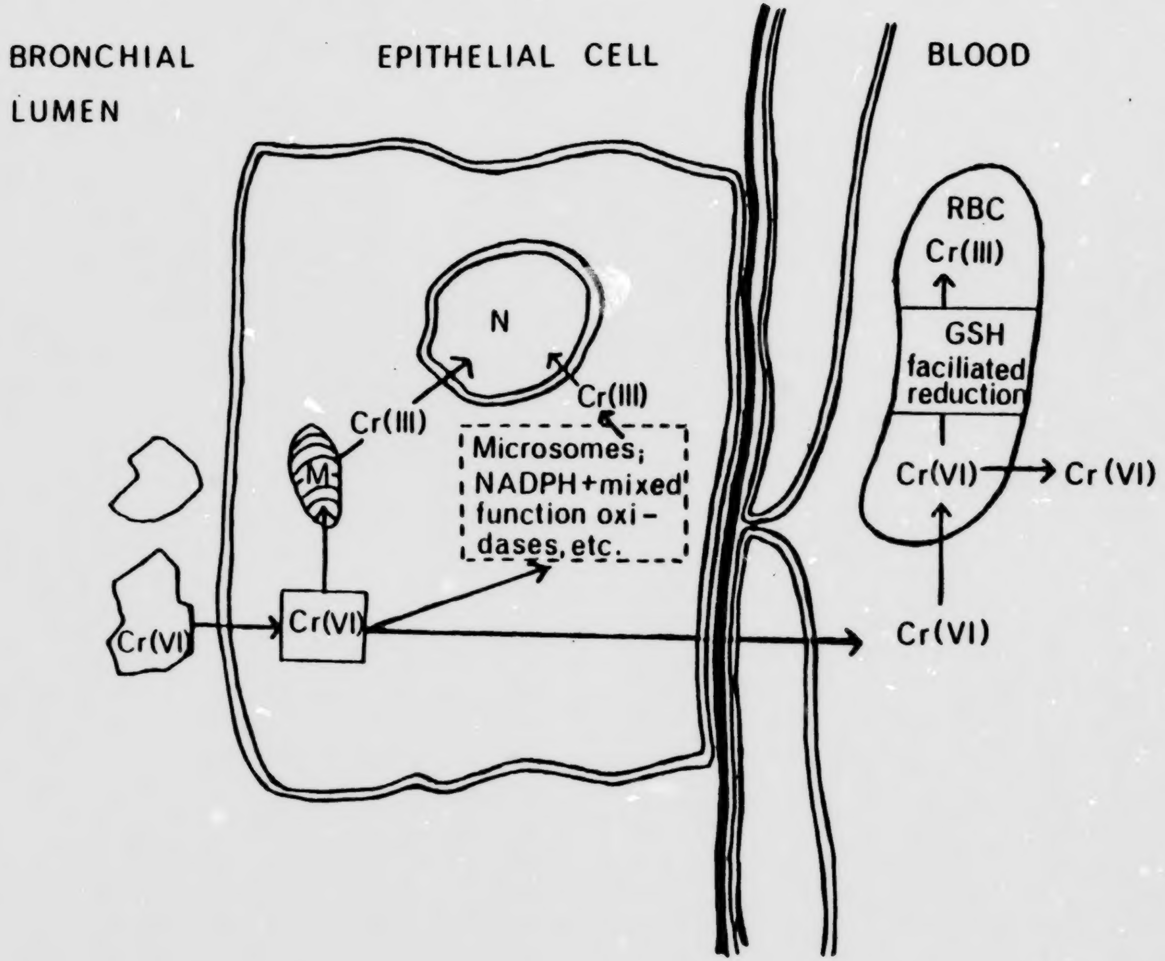
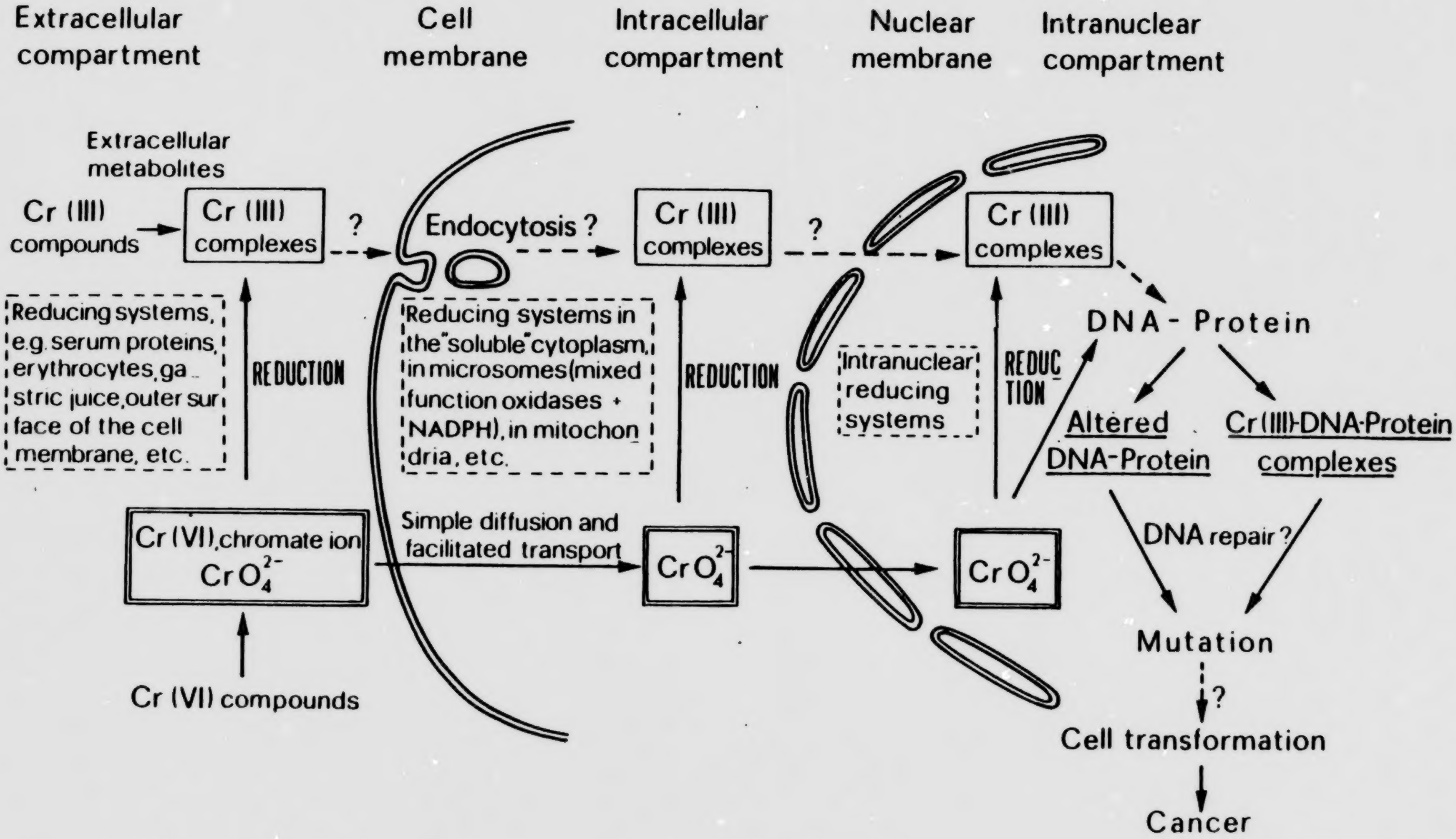

BRONCHIAL LUMEN
EPITHELIAL CELL
BLOOD
N
M
Cr(III)
Cr(III)
Microsomes; NADPH+mixed function oxi- dases, etc.
Cr(VI)
Cr(VI)
RBC
Cr(III)
GSH faciliated reduction
Cr(VI)
Cr (VI)
Cr(VI)

Figure 3.13 Possible mechanism of chromium mutagenicity and carcinogenicity. Reproduced with permission from Levis and Bianchi (1982).


Extracellular compartment
Cell membrane
Intracellular compartment
Nuclear membrane
Intranuclear compartment
Extracellular metabolites
Cr (III) compounds
Cr (III) complexes
?
Endocytosis ?
Cr (III) complexes
?
Cr (III) complexes
Reducing systems, e.g. serum proteins, erythrocytes, gastric juice, outer surface of the cell membrane, etc.
REDUCTION
Reducing systems in the "soluble" cytoplasm, in microsomes (mixed function oxidases + NADPH), in mitochondria, etc.
REDUCTION
Intranuclear reducing systems
REDUCTION
DNA - Protein
Altered DNA-Protein
Cr(III)-DNA-Protein complexes
Cr (VI), chromate ion $CrO_4^{2-}$
Simple diffusion and facilitated transport
$CrO_4^{2-}$
$CrO_4^{2-}$
Cr (VI) compounds
DNA repair ?
Mutation
?
Cell transformation
Cancer

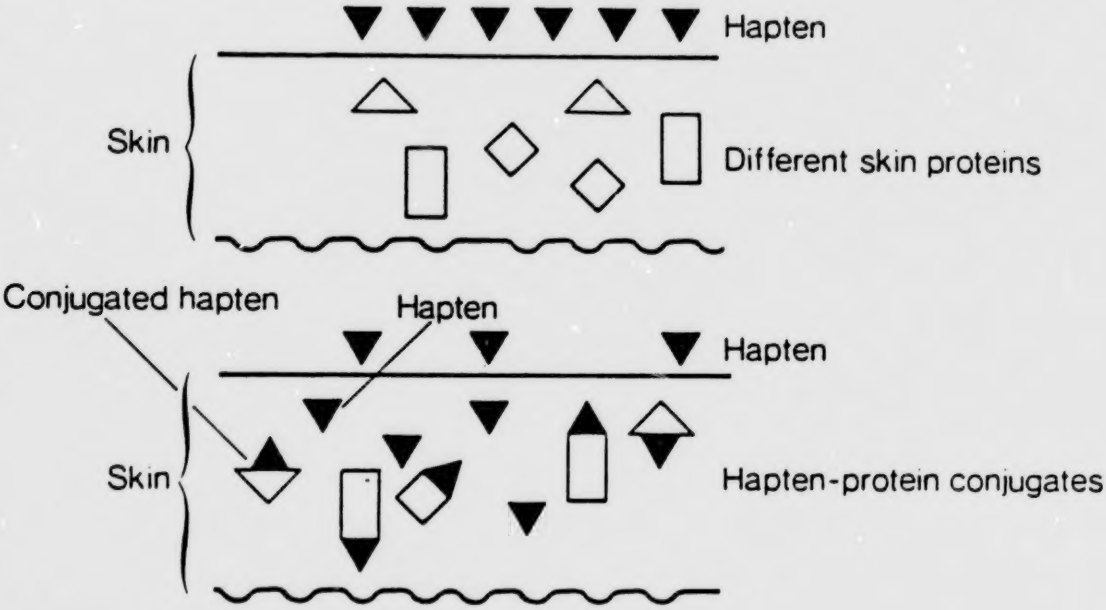

Hapten
Skin
Different skin proteins
Conjugated hapten
Hapten
Hapten
Skin
Hapten-protein conjugates

Figure 3.14 Antigen formation: an illustration of how low-molecular-weight substances, called haptens, can become immunogenic by combining with skin proteins subsequent to diffusion into the skin. Reproduced with permission from Polak (1983).

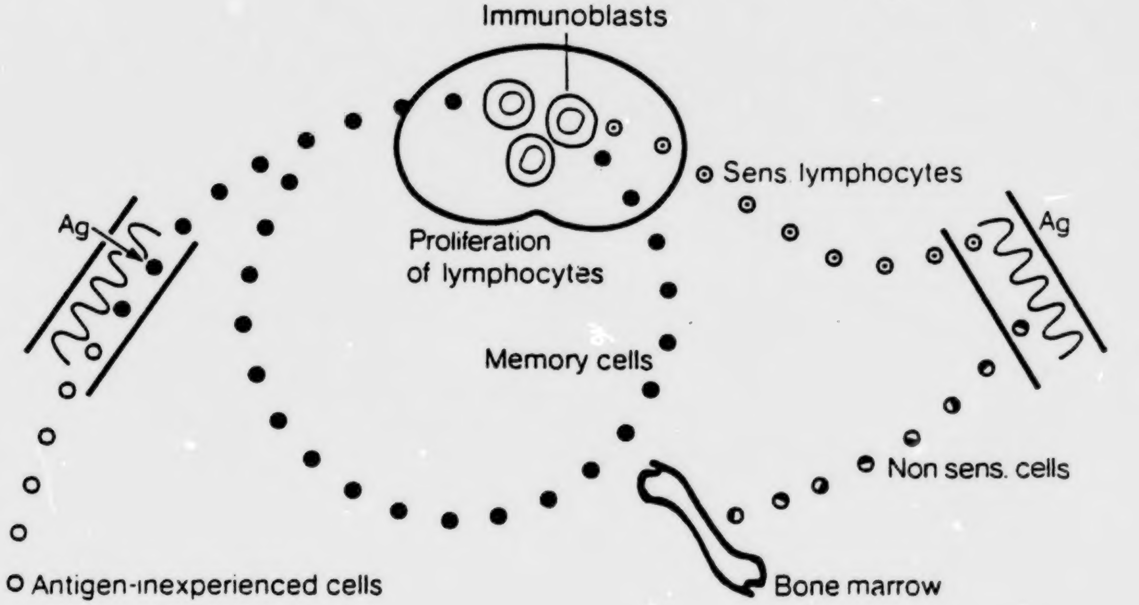

Immunoblasts
Ag
Sens. lymphocytes
Ag
Proliferation
of lymphocytes
Memory cells
Non sens. cells
Antigen-inexperienced cells
Bone marrow

Figure 3.15 A simplified illustration of the events believed to occur in contac sensitivity. Hapten-protein conjugates are recognized by antigen inexperienced specific lymphocytes (T-cells). These cells migrate to the draining lymph node, where they proliferate and differentiat into memory and effector cells. Sensitized lymphocytes, after leaving the lymph node, circulate in the body. A new contact with the specific antigen elicits an inflammatory skin reaction with the help of nonsensitized cells, secondarily involved in the eliciting phase. Macrophage-like Langerhans' cells are thought to participat in the formation of the antigenic complex, and appear important in both the induction and elicitation phases of contact sensitivity Reproduced with permission from Polak (1983).

## Table 3.1

Oxidizing Power of Chromate in Relation to pH[a]

| | E°' | pH |
|---|---|---|
| weakly oxidizing<br>^ | - 0.13 | 14 |
| | - 0.04 | 13 |
| | + 0.16 | 11 |
| | + 0.36 | 9 |
| | + 0.46 | 8 |
| | + 0.52 (0.31)[b] | 7.4 (physiologic pH) |
| | + 0.56 (0.36)[b] | 7 |
| | + 0.66 (0.54)[b] | 6 |
| | + 0.76 (0.66)[b] | 5 |

Basic solution:

$$CrO_4^{2-} + 4H_2O + 3\ e^- \rightleftarrows Cr(OH)_3(s) + 5\ OH^-\ (E^0 = -\ 0.13V)$$

| | E°' | pH |
|---|---|---|
| | + 0.79 | 4 |
| | + 0.93 | 3 |
| | + 1.07 | 2 |
| v | + 1.21 | 1 |
| strongly oxidizing | + 1.35 | 0 |

Acidic solution:

$$HCrO_4^- + 7\ H^+ + 3\ e^- \rightleftarrows Cr^{3+} + 4H_2O\ (E^0 = +\ 1.35V)$$

---

[a] The formal potential E°' (which refers to non-standard conditions) was calculated employing the Nernst Equation for the reactions shown. In basic solution, the reduction of $CrO_4^{2-}$ generates $OH^-$ ions against a gradient. This is costly from an energy point of view, thus destabilizing the Cr(III) state relative to the Cr(VI) state (as denoted by the negative E°' values). In acid solution, the reduction of $HCrO_4^-$ is accompanied by the consumption of $H^+$ ions, and therefore increased acidity pushes the reaction from left to right as written (as denoted by the positive E°' values). Supplemental explanatory information is provided in the legend to Fig. 3.2.

[b] Potential-pH equilibrium diagrams show that the hydrated $Cr^{3+}$ ion cannot exist above pH 4 because of hydrolysis (Cary, 1982). In the absence of chelating agents, $Cr(OH)^{2+}$ is the predominant species at pH 5 and 6, $Cr(OH)_2^+$ at physiologic pH values around 7, and $Cr(OH)_3$ at pH ≥ 8. For the E°' values in parentheses, this speciation and the presence of $HCrO_4^-$ were taken into consideration.

Table 3.2

Water Solubility of Metal Chromates[a,b]

| Compound | Molecular Weight (amu) | $g\ l^{-1}$ | $moles\ l^{-1}$ | Temp. (°C) |
|---|---|---|---|---|
| $ZnCrO_4$ | 181.4 | insol. | insol. | 25 |
| $Zn_x(CrO_4)_y(OH)_z$ (basic Zn chromates) | -- | slightly sol.[c] | slightly sol. | 25 |
| $PbCrO_4 \cdot PbO$ | 564.4 | insol. | insol. | 25 |
| $PbCrO_4$ | 323.2 | $5.8 \times 10^{-4}$ | $1.8 \times 10^{-6}$ | 25 |
| $Pb_2(OH)_2CrO_4$ | 564.4 | 0.14 | $2.5 \times 10^{-4}$ | 17 |
| $BaCrO_4$ | 253.3 | $4.4 \times 10^{-3}$ | $1.7 \times 10^{-5}$ | 28 |
| $SrCrO_4$ | 203.6 | 1.2 | $5.9 \times 10^{-3}$ | 15 |
| $K_2Cr_2O_7$ | 294.2 | 49<br>(1020) | 0.17<br>(3.5) | 0<br>(100) |
| $CaCrO_4 \cdot 2H_2O$ | 192.1 | 163 | 0.85 | 20 |
| $K_2CrO_4$ | 194.2 | 629 | 3.2 | 20 |
| $Na_2CrO_4$ | 162.0 | 873 | 5.4 | 30 |
| $Na_2Cr_2O_7$ | 262.0 | 2380 | 9.1 | 0 |

[a] From Weast (1973) and IARC (1980).

[b] When values are calculated from the solubility products quoted in Sillén and Martell (1964), there are some discrepancies.

[c] Likely to be the order of 1 $g\ l^{-1}$.

## Table 3.3

### Measures to Assure Low Blank Values[a]

| Measure | Rationale |
|---|---|
| 1. Assure cleanliness of work area | To avoid obvious contamination |
| 2. Wearing of talc-free disposable gloves | Sweat and talc are known sources of metals |
| 3. Acid-wash glass and plastic wares | To prevent leaching and adsorption of metals |
| 4. Contamination-free specimen collection | To eliminate additions during handling |
| 5. Sealed specimen storage | To avoid contact with ambient air |
| 6. Minimum sample size | To minimize additions and contact with laboratory ware |
| 7. Use high-purity reagents | To reduce addition of contaminants |
| 8. Minimize reagent additions | To reduce addition of contaminants |
| 9. Direct, small-scale analytical procedures | To avoid excessive handling and transfers; to minimize surface area contact with containers and ambient air |
| 10. If required, work in clean-air installations | To reduce blank values to acceptable levels |

[a] Reproduced with permission from Nieboer and Jusys (1983).

Table 3.4. Relationship Between Ambient and Urinary Chromium (for Welders) (Urinary chromium concentrations, y, are in $\mu g\ g^{-1}$ creatinine or $\mu g\ l^{-1}$; and air chromium levels, x, are in mg Cr $m^{-3}$.)

| Relationship | Equation | Correlation Coefficient | N |
|---|---|---|---|
| A. ΔCr(urine) *versus* Cr in air (water soluble) | | | |
| creatinine corrected | $y = 3 + 202x \pm 9$ | 0.95 | 27[a,i] |
| creatinine corrected | $y = 2 + 198x$ | 0.92 | 6[b,c,i] |
| specific gravity corrected | $y = -11 + 236x \pm 23$ | 0.82 | 27[a] |
| neat | $y = -21 + 335x \pm 56$ | 0.65 | 27[a] |
| B. ΔCr(urine) *versus* Cr in air (total) | | | |
| creatinine corrected | $y = -12 + 179x \pm 13$ | 0.90 | 27[a] |
| creatinine corrected | $y = 2 + 140x$ | 0.89 | 19[d] |
| C. ΔCr(urine) *versus* Cr in air (water insoluble) | | | |
| creatinine corrected | $y = 15 + 51x \pm 30$ | 0.11 | 27[a] |
| D. Cr(urine) *versus* Cr in air (water soluble) | | | |
| creatinine corrected (end of shift) | $y = 25 + 265x \pm 20$ | 0.88 | 27[a,i] |
| creatinine corrected (end of shift) | $y = 6 + 466x$ | 0.96 | 6[b,e,i] |
| creatinine corrected (end of shift) | $(y = 26 + 100x)$ | 0.91 | 5[f,i] |
| neat (end of shift) | $y = 25 + 486x \pm 42$ | 0.85 | 27[a] |
| E. Cr(urine) *versus* Cr in air (total) | | | |
| specific gravity corrected (end of shift) | $y = 11 + 213x$ | 0.72 | 53[g] |
| neat (end of shift) | $y = 14 + 198x$ | 0.68 | 53[g] |
| neat (end of shift) | $(y = 22 + 400x)$ | | 23[h] |

Table 3.4 (continued)

a. From Tola et al., 1977. ΔCr(urine) refers to difference in urinary concentration between afternoon (1600) and morning (0700) specimens. Nearly all the water soluble chromium was Cr(VI). Reference specific gravity was 1.018.

b. From Mutti et al., 1979.

c. In another group of workers (N = 8) the relationship was $y = 0.03 + 86\ x$. This group was said to have lower chromium clearance, but see earlier comments in Section 3.4.3.

d. From Welinder et al., 1983.

e. In another group of workers (N = 8) the relationship was $y = 3 + 98\ x$. See footnote c for additional comment.

f. From Rahkonen et al., 1983. These data exhibit wide scatter.

g. From Sjögren et al., 1983. End-of-shift, afternoon urine specimens were collected. Reference specific gravity was 1.024.

h. From Gylseth et al., 1977. The equation was estimated from a non-linear log plot in the original publication. Because of the large volume of urine samples analyzed, the large amounts of reagent addition, and the prolonged treatment of the sample, these results are judged to be unreliable.

i. Very recently, Mutti et al. (1984) have reported the following relationships for 9 workers (6 welders, 2 chrome platers, and 1 chromate worker): $y = 4 + 167x$, $r = 0.88$, $N = 8$, $y$ = ΔCr(urine, creat. corr.), $x$ = water soluble Cr(VI); and $y = 11 + 384x$, $r = 0.88$, $N = 9$, $y$ = Cr(urine, creat. corr.), $x$ = water soluble Cr(VI). The end-of-shift value and the daily increase in urinary chromium corresponding to the 50 μg $m^{-3}$ TLV were estimated as 30 and 12 μg $g^{-1}$ creatinine respectively (cf. predicted value for incremental increase of 10 μg $g^{-1}$ in Table 3.5).

Table 3.5 Predicted End-of-Shift Urinary Chromium Concentration Increase in Relation to Water-Soluble Ambient Cr(VI) Concentrations

| Ambient Cr(VI) Concentration (mg Cr $m^{-3}$) | Predicted Incremental Increase in Urine Chromium Levels | |
|---|---|---|
| | Creatinine corrected[a,b,c] ($\mu g\ g^{-1}$ creatinine) | Neat[b,c] ($\mu g\ \ell^{-1}$) |
| 1.00 | 200 | 300 |
| 0.50 | 100 | 150 |
| 0.10 | 20 | 30 |
| 0.05[d] | 10[f] | 15 |
| 0.025[e] | 5 | 7.5 |
| 0.02 | 4 | 6 |

[a] $\Delta$Cr(urine; μg per g of creatinine) = 200 Cr (air; mg $m^{-3}$).

[b] $\Delta$Cr(urine; μg $\ell^{-1}$) = 300 Cr (air; mg $m^{-3}$), based on the expression in footnote(a) and an urinary creatinine concentration of 1.5 g$\ell^{-1}$ at an excretion rate of 1 ml $min^{-1}$ (Nieboer *et al*. 1983a).

[c] To convert μg Cr to μmol Cr divide by 52.0.

[d] ACGIH recommended TWA.

[e] NIOSH recommended TWA.

[f] See footnote i, Table 3.4.

Table 3.6 Urinary Chromium as an Index of Exposure for Chromeplating Workers[a]

| Type of plating | Water-Soluble Ambient Cr(VI) (mg Cr $m^{-3}$) | N[b] | Urinary Chromium (µg $g^{-1}$ creatinine) | | |
|---|---|---|---|---|---|
| | | | Observed[c] | N[b] | Predicted[d] |
| Hard | 0.06 ± 0.09 | 17 | 18 ± 16 | 21 | 18 |
| Bright | 0.006 ± 0.006 | 9 | 5 ± 3 | 13 | 4.5 |

[a] From Guillemin and Berode, 1978.

[b] N, number of data points averaged (separate specimen collections).

[c] End-of-shift specimens.

[d] Calculated from: Urine (µg Cr $g^{-1}$ creatinine) = 250 Air (mg Cr $m^{-3}$) + 3. The relationship reported by Lindberg and Vesterberg (1983) appears unlikely because it implies that daily urinary excretion exceeds uptake by a factor of about four, namely, Urine (µg Cr $\ell^{-1}$) = 4000 Air (mg Cr $m^{-3}$) - 2.

Table 3.7 Chromium Contents of Tissues at Necropsy of Occupationally Exposed Individuals[a]

| Type of Worker | Tissue with Elevated Chromium Concentrations[b] | Other Tissues Examined[c] | Reference |
|---|---|---|---|
| chromate manufacture | lung | more than 20: liver, kidney, lymph nodes etc. | Mancuso and Hueper, 1951 |
| chromate manufacture | lung & other respiratory tract components; lymph nodes | - | Baetjer *et al.*, 1959 |
| chromate manufacture | lung | - | Mancuso, 1975 |
| chrome plater | lung | - | |
| chromate manufacture | lung; adrenal | 10 others: brain, liver, kidney etc. | Hyodo *et al.*, 1980 |
| chromate manufacture | lung, lymph node | - | Tsuneta *et al.*, 1980;<br>Tsuneta, 1982 |
| chromate manufacture[d] | lung, lymph node | liver, spleen, kidney, heart | Teraoka, 1981 |
| chrome plater[e] | lung, lymph node | liver, spleen, kidney, heart | |

[a] All individuals died of lung cancer, unless otherwise specified.

[b] A finding was considered reliable if the chromium concentration exceeded that of the control by at least a factor of 10.

[c] Accumulations in some of these tissues appeared to have occurred, but the analytical protocol described does not permit a firm conclusion (see text).

[d] Three workers: one died of stomach cancer, one of esophagus cancer and the other of lung cancer

[e] Two workers: one died of lung cancer, and the other of pancreas cancer and metastatic lung cancer.

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# 4. CARCINOGENICITY OF CHROMIUM COMPOUNDS

## 4.1 Overview

It has been over half a century since a link was first suggested between occupational exposure to chromates and increased risk of lung cancer (Lehmann, 1932), and almost a century has passed since Newman (1890) reported a case of nasal cancer in a chrome pigment worker. Several further reports (e.g., Alwens et al., 1936 and Pfeil, 1935) prompted the German health authorities to accept lung cancer in chromate-producing workers as an occupational disease in 1936 (Langård, 1983). Ten years later in the U.S.A. lung cancer was officially accepted as being caused by chromate exposure (Hueper, 1966). Epidemiological studies conducted in numerous countries, including the U.S.A. (e.g., Machle and Gregorius, 1948; Mancuso and Hueper, 1951; Mancuso, 1975; Taylor, 1966; Hayes et al., 1979), Great Britain (e.g., Bidstrup and Case, 1956; Royle, 1975a,b), Scandinavia (e.g., Langård et al., 1980), the Netherlands (e.g., Frentzel-Beyme and Claude, 1980), Italy (e.g., Franchini et al., 1983), the U.S.S.R. (e.g., Pokrovskaya and Shabynina, 1973) and Japan (e.g., Satoh et al., 1981; Ohsaki et al., 1978) have since confirmed the existence of an increased cancer risk in association with exposure to chromium-containing compounds. Studies on the high incidence of lung cancer in workers exposed to chromates are still, in fact, appearing (e.g., Langård and Vigander, 1983 and Franchini et al., 1983).

Extensive reviews on the carcinogenicity of chromium have been published by government bodies, international organizations and academic institutions (e.g., IARC, 1973, 1980; NAS, 1974; NIOSH, 1973, 1975) and by individuals (e.g., Sunderman, 1976; Langård and Norseth, 1979; Norseth, 1979, 1981; Hayes, 1980, 1982; and Langård, 1983); the U.S. EPA is also preparing a reappraisal of the literature. A review of the epidemiological evidence was conducted by Enterline (1974) and the state of knowledge on the mutagenicity of chromium compounds was recently reviewed by Leonard and Lauwerys (1980) and Levis and Bianchi (1982).

Although exposure to chromium in a variety of different forms and occupational settings has been linked in numerous epidemiological studies to increased cancer risk (see Section 4.4), attempts to identify the specific causative agents of chromium-associated lung cancer by epidemiological methods have generally not been successful. The reasons for this include the fact that workplaces are often contaminated with a variety of trivalent and hexavalent chromium compounds as explained in Section 3.1 and again outlined in Section 4.4. Consequently, laboratory studies have played a major role in evaluating the carcinogenic potential of various chromium compounds. Important advances in the understanding of chromium carcinogenicity have also emerged from recent studies of mutagenic activity in bacteria and from work with isolated cell systems.

We will begin this chapter by summarizing the knowledge concerning the mechanism of chromium mutagenesis, as derived from in vitro studies (Section 4.2). The information obtained from animal experiments will be presented in Section 4.3, and we will discuss the epidemiological evidence on chromium and cancer in Section 4.4.

A differentiation was made by NIOSH (1975) between "carcinogenic" and "non-carcinogenic" chromates. Highly soluble hexavalent chromium compounds, including the monochromates and dichromates of hydrogen, lithium, sodium, potassium, rubidium, cesium and ammonium, as well as chromium trioxide, were designated as "non-carcinogenic". This distinction has generally been criticized, especially in the more recent reviews (e.g., Hayes, 1980; Langård, 1983; Langård and Hensten-Pettersen, 1983). While it was thought that slightly soluble chromates (hexavalent) were the carcinogenic culprits, current evidence (especially from in vitro mutagenicity studies and recent epidemiologic examination of chromium platers), suggests that highly soluble chromium(VI) compounds also have carcinogenic potential. On the question of whether or not trivalent chromium is carcinogenic we conclude that while hexavalent chromium carries a risk of a much greater order of magnitude, it would be incorrect to state that trivalent chromium is non-carcinogenic. Rather, we believe that the issue is one of intracellular bioavailability. And finally, although we provide some data to illustrate the dose-response relationship between chromium exposure and cancer risk, we join other authors (e.g., Langård, 1983; Hayes, 1980; Norseth, 1981) in stressing that the dose-response data is inadequate to allow an evaluation of risks at low levels of exposure.

These conclusions will be discussed more fully in Section 4.5 after the evidence on which they are based is presented.

## 4.2 Chromium Mutagenicity and Clastogenicity

Metals have long been known to exhibit mutagenic activity in isolated biochemical systems, in microbiological systems and *in vitro* cell systems (e.g., see Flessel, 1979; and Sunderman, 1979). Chromates have been specifically shown to cause mutations in *Escherichia coli* and *Salmonella typhimurium*, to influence DNA repair in *Bacillus subtilis* and to decrease the fidelity of DNA synthesis *in vitro* (see Norseth, 1981; Léonard and Lauwerys, 1980; Levis and Bianchi, 1982, for recent reviews of the many studies in this area).

It has been widely noted that the carcinogenicity of chromium seems to depend on the oxidation state of chromium and the water solubility of the compounds (e.g., Léonard and Lauwerys, 1980; Norseth, 1981; Langård, 1983). With respect to solubility, it is of considerable interest, however, that no significant differences seem to exist in dose-response between the soluble and sparingly soluble compounds in either the *E. coli* (Venitt and Levy, 1974) or *Salmonella* systems (Petrilli and de Flora, 1977, 1983). Potassium dichromate, a moderately soluble hexavalent salt (see Table 3.2), has been shown to be mutagenic in yeast (Bonatti *et al*., 1976) and soluble hexavalent salts have been shown by numerous investigators to induce morphological transformation and chromosomal aberrations in mammaliam cells *in vitro* (e.g., Nakamuro *et al*., 1978; DiPaulo and Casto, 1979; Tsuda and Kato, 1976; Fradkin *et al*., 1975). Chromosomal aberrations have also been found in workers exposed to potassium dichromate (Imreh and Radulescu, 1982) and chromic acid (Sarto *et al*., 1983). The mutagenicity of chromate-rich welding fumes has been documented as well (e.g., Hedenstedt *et al*., 1977; White *et al*., 1981; Andersen *et al*., 1983; Maxild *et al*., 1978). Interestingly, the mutagenic effects increased with the degree of water solubility of the chromium compounds in the fumes, and thus the Cr(VI) content (Hedenstedt *et al*., 1977; Sections 3.5.5 and 4.4.2.3). Figures 4.1 to 4.4 illustrate the number of mutagenic studies that have been conducted on the various chromium compounds; these are further summarized in Table 4.1 (also see Appendix I). Figures 4.1 and 4.2 show that the vast majority of studies on both soluble and sparingly soluble hexavalent compounds are mutagenic. As depicted in these figures, corroborating evidence from other in vitro tests (chromosomal aberrations, sister chromatid exchange, cell transformation, and DNA repair) is equally convincing.

With respect to trivalent chromium salts, it has been noted in the past (e.g., Norseth, 1981) that generally these compounds have little or no mutagenic activity in bacterial systems. In the experiments on E. coli conducted by Venitt and Levy (1974) only hexavalent chromium was shown to be mutagenic, with the trivalent chromium compound tested producing negative results. Recently Petrilli and De Flora (1983) again showed that 12 hexavalent chromium compounds were positive in the Ames test, while one compound with oxidation state 0 and seven trivalent compounds were negative, unless contaminated. Similarly, Venier et al. (1983) found hexavalent chromium but not trivalent chromium to be capable of inducing mutations or sister chromatid exchanges. Interestingly, Petrilli and De Flora (1977) actually found a deactivation of sodium dichromate as a mutagen in the Salmonella system by adding liver microsomal fractions. From the results of an extensive study of the mutagenicity of hexavalent chromium, Petrilli and De Flora (1978a) concluded that deactivation of mutagenic activity was due to reduction to the trivalent form. Further, they showed that oxidation of the trivalent form to the hexavalent form increased the mutagenic activity (Petrilli and De Flora, 1978b). Nishioka (1975) also found that the mutagenicity of hexavalent chromium disappeared when reduced to the trivalent form, in agreement with the report of Gruber and Jeanette (1978). In studies on hamster ovarian cells, Costa (1979) reported inhibition of DNA, RNA and protein synthesis by hexavalent chromium but not trivalent chromium. The mutagenic effect of potassium dichromate on hamster embryonic cells indeed disappeared when chromium(VI) reduced to the trivalent form (Fradkin *et al*., 1975). Although Nakamuro *et al*. (1978) reported that both hexavalent and trivalent soluble salts caused chromosomal aberrations in mammalian cells cultured *in vitro*, they concluded that the hexavalent salts were the most potent. Newbold *et al*. (1979) could not demonstrate this effect in response to a soluble trivalent salt (chromium(III) acetate).

Löfroth (1978), who found trivalent chromium to be mutagenic only at concentrations ten times higher than the hexavalent form, put forward the theory that the causative agent is trivalent chromium bound to genetic material after reduction of the hexavalent form by the NADPH-dependent microsomal enzyme system. He attributed the decreased mutagenic activity of trivalent chromium to decreased uptake of the trivalent form by the cells. Levis *et al*. (1977,1978), based on studies using hamster fibroblasts and human epithelial-like cells, suggest that the hexavalent form is reduced by passing the plasma membrane, but agree that the trivalent form is the active agent within the cell. Trivalent chromium salts have indeed been known to give positive results in various experimental model systems and are known to be capable of altering the physiochemical properties of macromolecules (see Sections 3.3.1 - 3.3.7). Sirover and Loeb (1976) found that both trivalent and hexavalent chromium increased error frequency in DNA synthesis _in vitro_. However, 16 mM of hexavalent chromium was required to produce the same effect as 0.64 mM of chromium(III). In studies by Levis _et al_. (1977,1978) both the hexavalent and trivalent salts decreased DNA synthesis. They found that after adding either form, only the trivalent species was found inside the cells. To produce equivalent effects, about a 100-fold less chromate was needed than of Cr(III). The impermeability of cell membranes to trivalent chromium complexes has actually been known for several decades (e.g., Gray and Sterling, 1950; Rajam and Jackson, 1958). Warren _et al_. (1981) however, showed that given the right ligand environment, trivalent chromium can cross the cell membrane. They indeed found that 4 of 17 trivalent chromium compounds tested were active in both an _E. coli_ differential repair assay and on the Ames test in _Salmonella typhimurium_; 4 others were positive only in the repair assay.

The number of studies revealing positive and negative results in mutagenicity and clastogenicity tests on various soluble and insoluble trivalent chromium compounds are illustrated in Figures 4.3 and 4.4, respectively, and are also summarized in Table 4.1. It thus seems that the greater importance of exposure to hexavalent chromium with respect to cancer risk may well relate to the ability of the chromate anion in passing biological membranes. Once inside the cell, the hexavalent form is rapidly reduced to the trivalent. An understanding of the mechanism of this process has been emerging (see Section 3.3.4.3). Experimental evidence exists that the electron-transport cytochrome P-450 system may be involved, and that the direct reduction by glutathione and other reducing agents also occurs. Norseth (1981) has concluded that the biological effects of chromium(VI) including its carcinogenic effects, are the result of this transformation, or the subsequent binding of the trivalent form to ligands within the cell, or both. Based upon the experimental evidence reviewed here and in Sections 3.3 and 3.4, a model of chromium uptake and mutagenicity/carcinogenicity is presented in Section 3.6 (also see Figs 3.12 and 3.13). As elaborated in Sections 3.6.2 and 4.5.1, intracellular bioavailability of the Cr(III) ion may be a critical determinant in the events leading to cancer.

## 4.3 Carcinogenicity Studies in Animals

Studies of chromium carcinogenesis in animals have been thoroughly reviewed and comprehensively tabulated in recent documents. The tables produced by IARC (1980) are included as Appendix II.

While observations in humans of a link between exposure to chromium compounds and increased risk of cancer had been reported since 1932 (see Section 4.4), early attempts to induce cancer in experimental animals (including mice, rats, guinea pigs, cats and rabbits) by inhalation or parenteral administration of metallic chromium, chromite ore, and various chromium compounds were largely unsuccessful (Lukanin, 1930; Akatsuka and Fairhall, 1934; Shimkin and Leiter, 1940; Schinz, 1942; Schinz and Uehlinger, 1942; Hueper, 1955; Baetjer et al., 1959a).

Hueper and his colleagues (Hueper, 1958; Hueper and Payne, 1959) reported the development of squamous cell carcinomas and sarcomas of the lung and fibrosarcomas of the thighs in rats who had received intrapleural or intramuscular implants of chromite ore. He showed that implantation of calcium chromate also produced sarcomas (ibid). Hueper concluded that chromium is carcinogenic when present in a form which provides for its adequate biological availability, which in turn, appears to depend on the degree of solubility of the chromium compound in the particular biological medium. Through a series of experiments with a variety of chromium compounds possessing a range of solubility in water and Ringer's solution (including calcium chromate, zinc chromate, strontium chromate, lead chromate and barium chromate), Hueper and Payne (1959) confirmed the validity of this solubility hypothesis. Subsequent studies (e.g., by Payne, 1960a; Dvizhkov and Fedorova, 1967; and Röe and Carter, 1969) confirmed that intraosseous, intramuscular, subcutaneous, intrapleural and intraperitoneal injections of Cr-metal powder and hexavalent chromium compounds can produce local sarcomas in rodents. Steffe and Baetjer (1965), however, were still unable to produce malignant tumours of the respiratory tract in rabbits, guinea pigs, rats or mice by administering various chromate materials by inhalation and/or intratracheal injection. Figures 4.5 to 4.10 illustrate the negative and positive findings for each group of chromium compounds tested by implantation, ingestion or injection (also see Appendix II). Negative studies in which the follow-up period was felt to be too short (i.e., less than 2 years) and positive studies in which the strain had a high incidence of spontaneous tumours were excluded from these figures. A perusal of the data indicates that very convincing evidence exists that calcium chromate (Fig. 4.5) and lead chromate (Fig. 4.6) are carcinogenic; while only a few studies have been carried out on zinc, strontium, and chromium chromates, these compounds also appear to be carcinogenic (Fig. 4.7). Clear trends are absent for other insoluble chromium compounds (Fig. 4.8) and chromium oxides (Fig. 4.9), and data for soluble forms of chromium (mostly hexavalent chromates) are essentially negative (Fig. 4.10).

As clearly shown in Figure 4.11 which depicts the inhalation studies, it has proven very difficult to demonstrate a carcinogenic response in the lungs of experimental animals. Thus the question of which compounds are the most potent respiratory carcinogens is still not resolved. In fact, the only study reported to demonstrate tumourigenic activity of a chromate with inhalation techniques was that of Nettesheim _et al_. (1971) in which adenocarcinomas and alveologenic adenomas (but no bronchogenic tumours) developed in mice exposed to chronic inhalation of calcium chromate (Fig. 4.11 and Appendix II). Tests in progress have suggested positive results for unspecified intermediates from chromate production as well (Hayes, 1979, as cited by Norseth, 1981). As noted by Langård (1983), inhalation exposure in animals, while constituting the experimental route of administration most representative of human exposure, is fraught with difficulties, both in the design of the experiments and in the interpretation of the results (see also Phalen, 1976; Campbell, 1976; Langård and Nordhagen, 1980). Therefore, Kuschner and Laskin (1971) developed a different method which consisted of intrabronchial implantation of stainless steel wire-mesh pellets as a carrier of different chromates. Using this method they succeeded in inducing squamous cell and adenocarcinomas in lungs of rats exposed to calcium chromate. By comparing the carcinogenicities of calcium chromate, chromic chromate, chromium(III) and chromium(VI) oxides they concluded that the moderately soluble hexavalent chromate (calcium chromate) was the most potent respiratory carcinogen. Levy (1975) also tested a number of chromates by this method. He found 7 bronchogenic tumours in 100 rats exposed to calcium chromates, 3 in 100 rats exposed to zinc potassium chromate and 0 in the control rats.

Little can be concluded from animal studies regarding the potential of trivalent chromium compounds to cause respiratory cancer in humans. Hueper (1961) did report a low incidence (3%) following chromium(III) acetate implantation (which was considered as negative for the compilation in Fig. 4.10), compared to a high incidence of respiratory tumours following intrapleural implantation of, for example, calcium chromate, strontium chromate and chromic chromate (57%, 49% and 74%, respectively in 35 rats tested). A similarly low incidence for chromium(III) acetate was later confirmed by Hueper and Payne (1962) (also reported as negative in Fig 4.10; also see Appendix II). While the small number of animals used by Hueper and Payne (1962) and the lack of detail in the report by Hueper (1961) make it difficult to evaluate the carcinogenicity of the compounds tested, these studies do suggest that of the two valences, hexavalent chromium seems to be more potent. Furthermore, ingestion of trivalent chromium acetate failed to induce tumours (Fig. 4.10; Schroeder _et_ _al_., 1964, 1965; Ivankovic and Preussman, 1975), although only low doses were tested. Hexavalent chromium has not been tested by this route. Although it can be said that not a single animal study has unequivocally demonstrated that metallic chromium or trivalent chromium is carcinogenic, as can be seen from Figures 4.5 to 4.11, it is also true that these compounds have been studied less extensively than many of the hexavalent chromium compounds.

One may conclude that there is certainly strong evidence that at least some chromium(VI) compounds are carcinogenic to animals when administered by certain routes. Generally, as illustrated in Figures 4.12 and 4.13, low-dose experiments tend to be negative and high-dose tests produce positive results. The data in Figure 4.13 suggest that the tumour yield increases with the dose of metal chromate administered (expressed in mg of chromium), independent of the metal cation ($Cr^{3+}$, $Pb^{2+}$, $Sr^{2+}$ or $Zn^{2+}$) and mode of administration. A threshold value between 2 - 4 mg Cr is suggested. However, the apparent dose-response relationship in Figure 4.13 must be viewed with caution as the number of data points for each metal chromate and mode of administration is few. Conceivably, each mode of injection and each type of metal chromate might generate individual responses. Furthermore, data for single and cumulative dose experiments were combined in the construction of Figures 4.12 and 4.13. Because of these inherent uncertainties, the apparent dose-response relationships shown must be considered of limited value in quantitative human risk assessments. Even the induction of respiratory tumours by Kuschner and Laskin (1971) is of limited usefulness, as intrabronchial implantation using a mechanical carrier is quite unlike exposures experienced by humans in occupational settings. For some chromium compounds, animal evidence provides the only point of reference because no epidemiological data exists on which quantitative assessments can be made. However, as will be shown in Section 4.4, for most chromium exposures considerable epidemiological evidence has accumulated. The animal data presented in this section has confirmed the carcinogenicity of sparingly soluble and moderately soluble hexavalent chromium compounds, and thus has contributed to knowledge on the potential role of solubility and valency. As we will see below, they also provide supportive evidence with respect to quantitative epidemiologic trends. The epidemiological data, which usually forms the cornerstone for dose-response assessments, will be reviewed below. An overview of the carcinogenicity of chromium compounds that considers all the evidence -- i.e., from bioassays, animal studies and epidemiologic reports -- is presented in Section 4.5.

## 4.4 Epidemiological Studies on the Carcinogenicity of Chromium

As mentioned in Section 4.1, epidemiological studies on the carcinogenicity of chromium have been carried out in numerous countries and have clearly documented the link between chromium and cancer. These studies are outlined in Tables 4.2 to 4.6. While the most extensive investigations have focussed on the chromate chemical producing industry, on chromate pigment production (Tables 4.3 and 4.4) and on chromium plating (Table 4.6), and which showed a positive link, studies on ferrochromium production (Table 4.5) and other secondary users of chromates are more equivocal. They, nevertheless, suggest that these workers may also be at increased risk of developing cancer. Furthermore, the possibility that exposure to chromium compounds might cause cancer at sites other than the respiratory tract has been raised in a number of reports (Tables 4.3 and 4.6). The most important issue, however, is the characterization of level and duration of exposure associated with cancer development in these studies, so that preventive measures may be rationally designed.

In order to appraise properly the results of 35 years of epidemiological investigation, certain methodological factors must be taken into account. The Advisory Council on Occupational Health and Occupational Safety has recently published a detailed memorandum to the Minister of Labour (ACOHOS, 1983) stipulating the principles and procedures for the interpretation of epidemiological studies. These criteria for evaluating historical prospective and proportional mortality studies outlined are reproduced in Appendix III, and have been adopted in this report (c.f., Tables 4.3 to 4.6).

### 4.4.1 Studies in the Chromium-Producing Industries

#### 4.4.1.1 Production of monochromates and dichromates

Clinical observations from Germany in the 1930's strengthened earlier suspicions that workers in chromate production plants had an increased risk of developing lung cancer. (Baetjer in 1950 reviewed 122 cases of respiratory cancer among U.S. and German chromate workers that were reported before 1950.) Alwens and Jonas (1938), for example, reported 20 cases of lung cancer in dichromate-producing workers. The workers in the chromate industry were exposed to trivalent chromite ore as well as hexavalent chromium in the form of sodium chromate, sodium and potassium bichromates, calcium chromate and chromium(VI) oxide. No quantitative exposure data was provided, but analysis of the lungs of some of the cancer cases revealed high water/acid soluble and insoluble chromium contents (Baetjer _et al_., 1959b). As analytical methods were quite different then, no direct comparison can be made with more recent results (Langård, 1983; see Sections 3.5.2.5 and 3.5.4 and Table 3.7).

These early reports provided the impetus for a number of epidemiological studies in the chromate producing industry in the U.S., Great Britain and Japan. A list of the plants studied by the various investigators is provided in Table 4.2A, and the available information on exposure conditions in these plants is outlined in Table 4.2B. The reader is referred to Chapter 3 (Section 3.1 and Fig. 3.1) for a discussion of the chemical speciation of chromium encountered in industry. The methodological details and epidemiological findings by the various investigators are outlined in Table 4.3.

_U.S. Studies_: Machle and Gregorius (1948) studied 7 chromate plants in the U.S., for which adequate employment records existed in 6 (see Table 4.2). Their data showed that a similar high incidence of respiratory cancer existed in 5 of the plants assessed individually. Their overall lung cancer death figures represented an excess of 16 times the expected ratio. Some authors (e.g., Langård, 1983) express the opinion that this is an underestimate, as minimum initiation dose and latent period were not taken into consideration, retired workers were not included, nor was the reference population comparable. A slight increased risk of digestive cancer was also reported. The U.S. Public Health Service (Brinton et al., 1952; Gafafer, 1953) studied the same chromate plants. In this study, the cohort was limited to men who were members of a sick benefit association, membership of which was voluntary prior to 1949. Only those workers who died within one year after becoming disabled were included in this study. They reported a 4.5-fold increase in cancer at all sites and a nearly 29-fold increase in respiratory cancer. They found no excess risk for cancer at other sites. As shown in Table 4.3, the respiratory cancer risk was greatest for coloured males. Additional evidence supporting these observations comes from a case-control study of lung cancer in two Baltimore hospitals (Baetjer, 1950a,b), the details of which are reproduced in Table 4.3.

Mancuso and Hueper (1951) studied chromium-induced lung cancer in the Ohio plant, using proportionate mortality in their analyses. They found 6 respiratory cancer deaths in exposed workers, and an observed/expected ratio for lung cancer of 15. The available exposure information for this plant, as provided in Table 4.2B, showed that workers were exposed to three distinct types of chromium compounds: (1) the insoluble crude or chromite, which contains trivalent chromium [Cr(III)]; (2) the water-soluble hexavalent sodium monochromate (the intermediate formed after the roasting treatment); and (3), the water-soluble sodium dichromate end product which again has chromium in the hexavalent state [Cr(VI)] (see Fig. 3.1). Average frequency distributions of these fractions in air samples for various plant operations in six of the U.S. plants are also reproduced in Table 4.2B. Based on the hygiene assessments conducted by Bourne and Yee (1950), Mancuso and Hueper (1951) indicated that approximately 96% of the workers were predominantly exposed to water-insoluble chromium(III) (chromite), with no workers exposed exclusively to hexavalent chromium. Mixed exposures thus occurred, which is typical of chromate production facilities. Mancuso and Hueper's weighted average exposure estimates for the men who died of lung cancer range (in mg Cr $m^{-3}$): 0.01 - 0.15 water-soluble chromium, 0.10 - 0.58 for insoluble, and 0.11 - 0.66 for total chromium. These air levels fall within the ranges reported by Gafafer (Table 4.2B). On analysis of tissue from one worker who died of lung cancer and one who died of bladder cancer, significant amounts of chromium were found in the lungs (26 - 46 $\mu g\ g^{-1}$ compared to 0.3 $\mu g\ g^{-1}$ for controls; wet wt). Mancuso and Hueper believed this to be consistent with the high cancer rate observed. Thus, while postulations concerning the etiology of lung cancer had centered on hexavalent chromium, this study emphasized the latent importance of the insoluble trivalent chromium as potentially carcinogenic. Mancuso (1975) presented a further report, detailing findings from the 1931-1937 part of the cohort, followed up until 1974. Again using hygiene data collected in 1949, the time-weighted average of exposure to insoluble, soluble and total chromium (per $m^3$) was calculated for each occupation and for each worker in the plant. From detailed work-history records, duration of exposure in each job was established, thereby allowing the calculation of exposure years to each type of chromium for each worker. His data suggests that the age-adjusted lung cancer death rates per 100,000 increased with both insoluble and total chromium exposures. Mancuso, however, also noted that for a given exposure to insoluble chromium, an increase in exposure to total chromium resulted in an increased death rate from lung cancer; whereas for a given exposure to total chromium, increasing exposure to insoluble chromium led to a decreasing lung cancer death rate. He concludes that the data is consistent with the lung cancer risk being a function of both the water-soluble [mostly Cr(VI)] and water-insoluble chromium [mostly

Cr(III)]; i.e., the total chromium rather than to one class of compounds. On this basis, Mancuso (1975) articulated the controversial conclusion that "carcinogenic potential extends to all forms of chromium". IARC (1980) agreed that the data demonstrated a dose-response effect for lung cancer death with increasing chromium exposure indices, and that both soluble and insoluble chromium appeared to be implicated in carcinogenesis. However in their view, Mancuso's generalized conclusion that all chromium compounds must be regarded as suspect was not justified by his data.

In 1966 Taylor had presented an extensive follow-up study of chromate workers who had been employed in one of three U.S. chromate-producing plants between 1937 and 1940. Information on deaths was derived from Old-Age and Survivor Disability Insurance, and latency period was not subtracted when calculating the expected death figures. Nonetheless, chromate workers were found to have an excess of respiratory cancer of 8.5 times the expected rate. The only measure of exposure to chromate dust used in this study was duration of employment. Taylor (1966) did indeed demonstrate a dose-response relationship with this parameter. The observed-to-expected ratio varied from 4 to 18 with increasing duration of work. Deaths due to other cancers were also slightly increased. Enterline (1974) recalculated the data obtained by Taylor (1966), using different values for expected incidence, and he reported a

9.4-fold increase in respiratory cancer for the entire 20-y observation period. Enterline also specifically referred to the great excess in neoplasms of the mucocilliary sinuses and to the slight excess in digestive cancers. Moreover, he noted that the greatest risk was in the period immediately after the cohort was identified; and concluded that the rather short latency period observed in this and previous studies probably reflected exposure to a very potent carcinogen.

Hayes et al. (1979) conducted a well-designed study of dichromate-producing workers from the Baltimore plant in order to determine if improvements in the hygienic standards introduced in the U.S. chromate-producing industry in the early 1950's had eliminated the increased cancer risk. The cohort was divided into a "high exposure" group consisting of workers who had been employed in the old plant or whose exposure was undetermined, and a "low exposure" group consisting of workers employed only in the new plant since 1950. The workers were further sub-divided into those employed from 1945 to 1949, from 1950 to 1959, and from 1960 onwards. Although there was no information in the report on actual exposure levels, there was an apparent dose-relationship as associated with length of employment in the 'high exposure' group. No lung cancers were observed in the most recently hired group, as would be expected, since they had not been under observation long enough to develop chromate-induced cancer. In the other two sub-groups, the observed/expected ratio for "high exposure" workers varied between 1.8 and 3.4 with no significant differences between them. Hayes et al. (1979) concluded that the risk of cancer was not eliminated in the new plant, as the relative risk for "low exposure" workers was 4.0. They also pointed out that even short-term workers, employed 3 months to 2 years, experienced a higher lung cancer mortality than expected. Further, these authors noted that employees at the "wet end" of the production process, whose exposure was largely to highly soluble chromate compounds, had the highest cancer risk. As pointed out, and as already alluded to, many of these workers had mixed work exposures.

Hill and Ferguson (1979) investigated the same Baltimore, Md., plant. In contrast to the comprehensive study by Hayes et al. (1979), they employed the statistical method of "probability window analysis" to demonstrate trends in lung cancer risk in association with the modernization of the plant. A statistically significant decline was found from 23 to 7 lung cancers in 24-y time periods covering the intervals 1927-1951 and 1952-1976, respectively. IARC (1980) stresses that no conclusions on improved safety in the plant can be drawn, as the method of analysis compares only lung cancer cases, not rates of lung cancer, and does not make allowance for the latency period.

British studies: Bidstrup (1951) and Bidstrup and

Case (1956) studied the risk of lung cancer in chromate-producing plants in England. In the early survey (Bidstrup, 1951), only one cancer was detected from chest X-rays performed on 724 workers in three chromate plants. In the follow-up of these workers (Bidstrup and Case, 1956), 12 lung cancer cases were observed, while only 3.3 were expected for an increase of 3.6-fold as shown in Table 4.3. Langård (1983) has noted that if all of the lung cancer cases occurred in the group of 237 workers who had been employed for more than 15 years, only this group should have served as the base for estimation of expected cases and a larger increased risk would have resulted. From the exposure data (collected in 1949) included in Table 4.3, it may be seen that mixed exposure to soluble Cr(VI) and insoluble Cr(III) occurred for all operations in the British plants; a situation similar to that described earlier for the American counterparts.

Recently, Alderson *et al*. (1981) followed up this British chromate-producing population, and included workers employed in subsequent years to assess the effect of improvements in plant hygiene. They found 116 lung cancer cases while 48 were expected, for an overall relative risk of 2.4. They also reported two cases of nasal cancer. The relative risk for developing lung cancer decreased from 3.0 before plant modification to about 1.9 afterwards, which the authors noted failed to reach statistical significance but indicated an appreciable reduction of the lung cancer risk. No quantitative industrial hygiene data were provided.

Japanese Studies. Ohsaki et al. (1978) studied the occurence of lung cancer in a small cohort of active and retired dichromate and chromic acid production workers. As shown in Table 4.3, they found 14 lung cancer cases, which was calculated to be more than 50 times the expected figures. Although no quantitative exposure data was given, the large number of workers with nasal perforations, combined with the fact that the plant was old and there were few industrial hygiene measures enforced, suggested that chromate exposure was likely very high. Recently in an update, Tsuneta (1982) reported the incidence of lung cancer in this plant as 16 times that of the general population. The histological type observed in their case series study was mostly squamous and small cell carcinoma. There were many heavy smokers among the cases. However according to Ohsaki et al. (1978), differences in smoking histories could not explain the very high risks observed (also see Abe et al., 1982).

Sano and Mitohura (1978) reported that 19 of the 36 deceased chromate workers in Tokyo died of cancer of the respiratory tract, and this was interpreted as evidence of a high risk of cancer associated with the chromate-production industry. Furthermore, it was reported that cancers of other organs might be caused by engaging in chromate work (Sano and Ebihara, 1979). By contrast, Satoh et al. (1981) who found a 9.5-fold increase in respiratory cancer among workers in a Tokyo chromate manufacturing plant, failed to note increases in cancers of other sites.

In summary, the studies of the chromate manufacturing industry have consistently indicated a strong association between exposure in this industry and increased risk of respiratory cancer. Often the relative risk has been found to be extremely high. Lack of exposure data precludes the establishment of a rigorous generalizable dose-response relationship. Because mixed exposure occurred in all plants studied, it is unclear whether trivalent chromium or hexavalent or both have an etiological role. Neither can the relative potencies of soluble versus insoluble hexavalent chromium be determined from these data, although both have been implicated.

### 4.4.1.2 Chromate pigment production

Although the chromate chemical industry has been the most extensively studied, the earliest reports of cancer related to chromium exposure stemmed from chromium pigment exposure (Newman, 1890; Gross and Kolsch, 1943; Baetjer, 1950a,b).

Langård and Norseth (1975) published the first epidemiological study linking chromium pigment manufacturing to lung cancer. They investigated a small work force exposed to lead chromate, zinc chromate, sodium dichromate and other substances in three Norwegian pigment plants. Three lung cancers were identified in the 24 workers who had been exposed for more than three years before 1973, for a relative risk of 38. As indicated in Table 4.4, chromium exposure at the time of the epidemiological study showed that workers were exposed to significant amounts of chromates (of sodium and zinc). Levels in the past were estimated to be similar, but exposure was to both zinc and lead chromates. A slight excess in cancer of the gastrointestinal tract was also observed in these workers (Langård and Norseth, 1979). The follow-up study (Langård and Vigander, 1983) reported three additional lung cancer cases for a relative risk of 44. As high as this is, Langård and colleagues (Langård and Anderson, 1980; Langård, 1983) felt that the reported relative risks are an underestimate. Langård (1983) noted that except for one of the cases, these cancer patients had been exclusively exposed to zinc chromate, implicating this pigment as the most likely causative agent in this study.

In 1976 the American Dry Color Manufacturers' Association commissioned a study of three U.S. plants which mainly produced lead chromate (EEH, 1976). The results, as shown in Table 4.4, indicate a considerably lower cancer risk than was seen in the Norwegian population. The authors felt that their findings remained consistent with the hypothesis that lead chromate is a carcinogen. The excess was found generally in long-term workers, albeit when excluding those exposed also to zinc chromate, the excess was based on only three deaths. In any case, they noted that due to the small number of deaths the relative excess of respiratory cancer could not be determined precisely. The authors also found an SMR of 778 for stomach cancer in the plant with exposure to both lead and zinc chromate, but felt that no causative link was yet warranted.

Davies (1978,1979,1984a,b) studied three chromate pigment plants in Great Britain. As shown in Table 4.4, an elevated risk of lung cancer was found only in the plants which produced both zinc and lead chromate, and was absent in workers who were employed only in the manufacture of lead chromate. Davies concludes: "the results provide no indication that lead chromate induces lung cancer in man" and "exposure which is relatively mild or lasts less than a year may not constitute an effective risk".

A study including 7 factories in 4 European countries was conducted in order to assess further the possibility of a cancer risk in workers exposed to lead chromate (Frentzel-Beyme and Claude, 1980). A moderately but consistently increased risk of lung cancer and other respiratory tract cancers was found. Unfortunately, almost all of the workers had also been exposed to zinc chromate. Similarly in the recent report by Sheffet _et al_. (1982) of increased cancer risk at a pigment plant (see Table 4.4), exposure was primarily to lead chromate but zinc chromate was also present.

In 1983 the American Dry Color Manufacturers' Association (Cooper, 1983) presented the findings of a further 5-year follow-up of their previous cohort and gave an indication of the exposure levels (see Table 4.4, footnote b). No evidence was found to support their earlier hypothesis that lead chromate was carcinogenic as no additional lung cancer deaths were observed in this sub-group (plant 1 workers). In contrast, for workers with mixed exposure (plants 2 and 3), a statistically significant elevated risk of lung cancer was again found, supporting the hypothesis that zinc chromate is carcinogenic. Note that exposure in plant 2 was not to lead chromate alone as believed in the 1976 study (see Table 4.4). An increased risk of stomach cancer was also again noted.

It thus seems that exposure to chrome pigments definitely increases the risk of respiratory cancer and possibly other cancers, such as stomach cancer. While it is not yet possible to differentiate conclusively between the cancer risk associated with zinc chromate as oppposed to lead chromate or other pigments, the evidence suggests zinc chromate is the most potent carcinogen in this group.

### 4.4.1.3 Ferrochromium-producing industries

While exposure in the manufacturing of chromates and chrome pigments is predominantly to hexavalent chromium, workers employed in ferrochromium production are primarily exposed to trivalent chromium (chromite ore is the starting material) and chromium metal. Typically, the ambient chromium is 10-30% water soluble. This fraction is largely hexavalent chromium (Langård et al., 1980; see Table 4.5 and Section 4.4.2.3).

Pokrovskaya and Shabynina (1973) studied workers employed in the production of chromium ferroalloys in the U.S.S.R. between 1955 and 1969, as shown in Table 4.5. Exposure to hexavalent chromium and benzo(a)-pyrene were noted as well as to trivalent chromium. The authors reported a relative risk for lung cancer of between 4.4 and 6.6. An increase in mortality from all malignancies and from esophageal cancer also occurred. Too few details were provided in the report to permit an evaluation of the validity of these findings.

Langård et al. (1980) studied a Norwegian ferrochromium and ferrosilicon production plant. A slight increase in lung cancer was demonstrated in workers who had been employed at the ferrochromium furnaces for many years, with the excess varying from 2.3 to 7.3 depending on the reference population used. Current exposure data for this cohort is reported in Table 4.5. The authors concluded that the role of trivalent chromium in the increase in lung cancer noted cannot be evaluated in view of the concomitant exposure to hexavalent chromium. They suggested, however, that the lower relative risk found in this study compared to studies in the chromate manufacturing industry may indicate that chromic compounds are less potent carcinogens than chromates.

Axelsson et al. (1980) studied the employees of a ferrochromium plant in Sweden. No increase in cancer-related deaths were noted among arc furnace workers who likely had the highest exposure to trivalent and hexavalent chromium, as shown in Table 4.5. These authors also concluded that the low incidence of lung cancer in this study reflected the lack of carcinogenicity of trivalent chromium. The different results obtained relative to the study by Langård _et al_. (1980) may relate to differences in statistical power, in cohort definition, in the observation period, or possibly in exposures to hexavalent chromium.

### 4.4.2 Studies in Chromium-Using Industries

#### 4.4.2.1 Chromium-plating industry

The chromium-plating industry has been in existence for a long time and has grown considerably in recent years (Hayes, 1980). While a number of case reports of lung cancer in chromium platers have been published (e.g., Barborik _et al_., 1958; Sennalova and Barborik, 1965; Michel-Briand and Simonis, 1977), the fact that this work tends to be done in a large number of small workplaces has not been conducive to epidemiological studies in this industry. Exposure in this setting is to dissolved hexavalent chromium trioxide (chromic acid) mist, which essentially contains chromate (as $CrO_4^{2-}$, $HCrO_4^-$, $H_2CrO_4$ or $Cr_2O_7^{2-}$ depending on pH, all hexavalent forms; see Section 3.2.2.1). Stern (1982) explains that the exposure to

Cr(VI) in plating shops depends strongly on the degree of ventilation available. With good local ventilation, ambient levels can be below 0.01 mg $m^{-3}$ compared to values near 1 mg $m^{-3}$ in its absence (also see Table 4.6). Floating plastic chips that top electrolysis baths can reduce the ambient levels significantly.

In 1975 Royle (1975a,b) conducted a historical retrospective as well as prospective mortality study of past and current chromium platers, as outlined in Table 4.6. The data indicate that the death rate for cancer of all sites was significantly increased in the exposed workers. Lung cancer and gastrointestinal cancer were also increased but failed to reach statistical significance. The results of the first two years of the prospective study demonstrated the same trend, but firm conclusions await the completion of this study.

Waterhouse (1975) presented preliminary results from a study of some 5000 chromium platers, of whom about half were defined as "chrome bath workers". About 80% of these workers were traced, and 49 lung cancer cases were observed with 35 expected. Although these preliminary results indicate a relative risk of only 1.4, the excess was found to be significant. Langård (1983) suggests that upon sub-dividing this cohort into different exposure categories and observation periods, it may be possible to determine dose-response relationships. Indeed, in a follow-up study of the non-bath workers in a large chromeplating plant, Waterhouse (1980) noted that when the cohort was subdivided into low exposure (0 - 2 y) and high exposure (3 - 8 y), there was a striking difference in cancer mortality, as shown in Table 4.6. For example, twelve cases of bronchial cancer were found in the low exposure group, exactly the number expected. In contrast, 10 cases were found in the high exposure group, compared to 3.3 expected.

Okubu and Tsuchiya (1977) studied cancer mortality in Japanese chromium platers, as shown in Table 4.6. The cohort was based on worker registration with an insurance society in 1970, and vital status was ascertained by questionnaire. No increased risk was found. However, the manner of defining the cohort, the possibility that the controls used may have also been exposed, and the incomplete ascertainment of vital status has led most researchers and organizations (e.g., Hayes, 1980; Langård, 1983 and IARC, 1980) to suggest that no conclusions can be drawn from this study.

Franchini _et_ _al_. (1983) recently reported their findings from a cohort of Italian chromium platers in three "hard" and six "bright" chromium electroplating plants. Eight cancer deaths were found in the cohort, against 4.2 expected. Statistical significance was not achieved, even when the latency period was specified to exceed 10 y (Table 4.6). In the subcohort of "hard" platers, who were exposed to roughly 10-fold the levels chromium(VI) compared to the "bright" platers (see Stern, 1982), 7 cancer deaths occurred against 2.7 expected. Three lung cancers were found, all in the subgroup of "hard" chromium platers; 0.7 were expected (c.f. data in Table 4.6 for latency > 10 y). These statistically significant results led the authors to conclude that exposure to water-soluble hexavalent chromium mist indeed increases cancer risk.

### 4.4.2.2 Spray-painting and related uses of chromium pigments

Chromate pigments are widely used in paints and inks. Dalager *et al*. (1980) conducted a proportionate mortality study on zinc chromate spray painters from two aircraft maintenance bases. They found 21 respiratory cancers compared to 11.4 expected. In addition, the risk appeared to increase with increasing duration of exposure. In contrast, Chiazze *et al*. (1980) in their study of automobile spray painters who had been exposed to chromate pigments for more than 5 years, failed to find an excess lung cancer risk. Differences in exposure levels, pigment composition, or personal protection could account for the discrepancies between these two studies. Definitive conclusions can therefore not be drawn about cancer risk for users of chromium pigments.

### 4.4.2.3 Stainless steel welding

It is now well known that welding fumes derived from stainless steel welding contain hexavalent chromium compounds (e.g., Fregert and Övrum, 1963; Naranjit *et al*., 1979, Thomsen and Stern, 1979). Evidence that stainless steel welders absorb chromium was presented in Section 3.5.3 (see also Gylseth _et al_., 1977; Tola _et al_., 1977 and Mutti _et al_., 1979; Welinder _et al_., 1983). _In vitro_ studies strongly indicate that stainless steel (SS) welding fumes generated by manual metal arc welding (MMA) contain mutagenic compounds (see also White _et al_., 1981; Maxild _et al_., 1978; Hedenstedt _et al_., 1977), and specifically that chromium(VI) compounds constitute the active component (e.g., Hedenstedt _et al_., 1977). Stern (1982) provides data illustrating that the SS/MMA combination yields the largest amount of water soluble Cr(VI). In his review of the mutagenicity studies of welding fumes, Stern (1980, 1981) concluded that the water-soluble hexavalent chromium, as well as the nickel, contained in welding fumes exhibited the same type of genotoxicity as is expected for pure fractions of $Ni^{2+}$ and chromate. He continues: "Since there is no evidence to the contrary, one should assume that the actual risk for genetic damage and other delayed health effects to welders can be expected to be similiar to that found in other industries with exposure to these substances".

Although welding is carried out on large scale in the industrialized world, few epidemiological studies have focussed on welders as a distinct group. Breslow _et al_. (1954) conducted a case-control study in which 10 welders were found among the patients with lung cancer against one of the controls. Milham (1976) noted a proportional mortality ratio of 119 for lung cancer among welders; Menck and Henderson (1976) demonstrated a standardized mortality ratio of 137 in their study. Beaumont and Weiss (1980) found 53 lung cancers against 40 expected in a group of welders; when taking into account a 20-y latency period the SMR increased from 131 to 169. Unfortunately, the type of welding performed by the workers in these studies was not identified. Similarly, Gottlieb (1980) found 8 lung cancer cases among welders against two among the controls, but again insufficient information was provided to implicate chromium exposure. Sjögren (1980), however, determined the mortality of 234 welders who had stainless steel welding as their major task for more than 5 years. Three lung cancer cases were found against 0.7 expected. Although the numbers were not large, the finding was significant ($p < 0.03$) and is suggestive of a chromium-related cancer risk. (The relative risk from nickel is thought to be much less than that for chromium.) In fact, Stern (1983a,b) recently reviewed 20 epidemiologic studies of lung cancer incidence among welders. His conclusions are summarized in the following quote from his paper (Stern, 1983a):

> "Analysis of epidemiologic studies of lung cancer among the general welding population indicates that the values of excess risk found can be accounted for by chance provided there is an average risk ratio of approximately 1.5, which almost completely can be accounted for by a general excess tobacco use, bystander exposure to asbestos predominantly among shipyard welders, and the presence in the total cohort of 5-10% stainless steel welders who are at the relatively high risk of a factor of 4-7 probably due to

their unique exposure to Cr and Ni."

### 4.4.3. Summary of Epidemiological Evidence

The risk of lung cancer has been consistently shown to be high in the chromate manufacturing industry, where exposure is to a mixture of insoluble (largely trivalent) and both moderately soluble and soluble (largely hexavalent) chromium compounds. The data suggest that even the more recently employed workers, who presumably are exposed to lower levels of chromium than workers employed previously, may have an excess cancer risk with prolonged employment.

In the chromate-pigment production industry, where the exposure is largely to sparingly soluble hexavalent compounds, high risk was also demonstrated in several studies. Moreover, one study indicated that the cancer hazard associated with production of chrome pigments may extend to the users of these pigments, such as spray painters.

In the ferrochromium producing industry, where workers are exposed to chromium which predominantly is metallic and trivalent, but nevertheless contains 10-30% soluble hexavalent chromates, the possibility of increased risk for lung cancer has also been raised. However, the relative risks reported were considerably lower than those generally experienced in chromate production. Since there have been conflicting reports, and in view of various methodological problems in the studies, the findings should be regarded as tentative.

Similarly, an increased risk of lung cancer has also been reported for chromium platers exposed primarily to highly soluble chromates. Again, the limited number of studies and limited strength and consistency of the association leave some room to question these findings, but the suggestion of a dose-response relationship in several of these studies (e.g. Waterhouse, 1980; Franchini _et al_., 1983) is certainly noteworthy.

Various studies have indicated that welders in general are at increased risk of cancer. Since there is as yet little epidemiological data on selected subcohorts of welders with restricted exposures, Stern (1980) stresses that supposition of process-dependent "hot spots" of risk must be sought on the basis of indirect (i.e., non-human) experiments. One recent epidemiological study (Sjögren, 1980) has indeed suggested that increased cancer risk is most strongly associated with stainless steel welding, specifically implicating chromium exposure as a causative factor. Stern (1983) estimates that the chromium induced risk ratio for respiratory tract cancer among stainless steel welders resulting from cumulative exposures of the order of 3 $(\text{mg m}^{-3})$ years may be near 6.

An excess of cancers at sites other than the respiratory system has been reported in chromate production workers (Machle and Gregorius, 1948; Enterline, 1974), chromate platers (Royle, 1975a,b; Waterhouse, 1980), chromate pigment producers (EEH, 1976; Langård and Norseth, 1979; Cooper, 1983; Davies, 1984a), and ferrochromium production workers (Pokrovskaya and Shabynina, 1973). Specifically, cancers of the gastrointestinal tract, as well as nasal and laryngeal cancers, have been noted to be slightly increased in various of these investigations (see Tables 4.3 to 4.6). Such findings, however, have not been consistently found in all studies, and no firm conclusions are possible. Interestingly, Hernberg *et al*. (1983) in a case-referent investigation of living patients with nasal and sinonasal cancers found a significant correlation ($p < 0.05$) with occupations in which there was suspected exposure to chromium (mostly welders).

## 4.5 Conclusions

### 4.5.1 On the Differentiation Between "Carcinogenic" and "Non-Carcinogenic" Chromium Compounds

In order to synthesize an overview and to formulate conclusions, the evidence from animal and biochemical studies, bioassays, epidemiological reports, and exposure data are first summarized and put into perspective.

Bioassays and related *in vitro* experiments indicate that all hexavalent chromates (both water-soluble and insoluble) can induce mutations and inflict chromosomal damage. In contrast, positive animal carcinogenicity data is only convincing for $CaCrO_4$ (intermediate solubility) and $PbCrO_4$ (sparingly soluble); and perhaps also for the sparingly soluble compounds $ZnCrO_4$, $SrCrO_4$ and $Cr_2(CrO_4)_3$, although only a few experiments have been reported for these. The results for soluble chromates are negative. For trivalent chromium, the animal studies show no consistent carcinogenic potential; and only when large concentrations are employed, or when a special ligand environment is generated around Cr(III), can mutagenicity be demonstrated.

While the Cr(III) ion and its complexes are generally excluded from cells, the chromate anion ($CrO_4^{2-}$) is readily taken up. By analogy to nickel compounds, crystalline forms of both Cr(III) and Cr(VI) may well gain access to cells by phagocytosis. This has recently been demonstrated for chromium(III) oxide ($Cr_2O_3$) in macrophages (Andersen, 1983). Of course, suitable intracellular dissolution must follow phagocytic accumulation for the toxicity to be expressed. Evidence is mounting that once inside a cell, the chromate anion is reduced to the Cr(III) ion. Because of its high charge (+3), and because it possesses almost inert (irreversible) kinetics, the Cr(III) ion has a great affinity for proteins, small chelating molecules (e.g., citrate) and nucleotides including DNA and RNA. Because of these interactions, intracellular accumulation of Cr(III) is believed to induce mutations and thus the Cr(III) ion is designated as the ultimate carcinogen (see Section 3.6.2 for details). And finally, intracellular bioavailability would appear to be a major determinant in chromium carcinogenesis. From this vantage point, hexavalent chromium compounds which generate the chromate anion have an edge in comparison to chromium(III) compounds which yield Cr(III) on dissolution, because a well-defined cellular uptake pathway exists for

$CrO_4^{2-}$ but not for Cr(III). Furthermore, the more insoluble chromates (compared to soluble chromates) have the potential of providing long-lived pools in the target organ, and there is some evidence that this occurs (see Sections 3.5.4 and 3.5.5.1). In contrast, exposure to soluble chromates are characterized by excretion components with relatively short biological half-times (matter of days), and on this account may not be able to exercise fully their carcinogenic potential.

The epidemiological data may be succinctly summarized as follows. Strong evidence for respiratory cancer has been established for chromate production and chromate pigment workers, with the data for chromeplaters being somewhat less certain; and that for ferrochromium manufacture and welders may be considered equivocal. Pigment workers were exposed mainly to the relatively insoluble chromates of zinc and lead (hexavalent chromium), and platers mainly to solutions of chromates (again hexavalent chromium). However, chemical production of chromates was associated with and characterized by mixed exposure. Workers were exposed to ambient materials containing chromite ore (a Cr(III) compound also containing Fe(II) as a major constituent), the moderately soluble $CaCrO_4$, and soluble sodium chromates (hexavalent; both mono and dichromates of sodium were handled). From this summary, it is evident that both relatively insoluble chromates (pigment workers) and soluble chromates (chrome platers) are implicated in chromium cancer etiology, while no clear judgement is possible about the relative carcinogenic potential or potency of the various compounds to which chromate production workers were exposed.

Proponents of the carcinogenicity of Cr(III) compounds fail to find strong support from animal studies and bioassays. Opponents of this view, suggest that the equivocal nature of the epidemiologic data for ferrochrome production workers, and perhaps also for welders, is evidence for the non-carcinogenicity of Cr(III)-containing substances. However, since only the sum of chromium metal ($Cr^0$) and Cr(III) has been determined in fumes associated with these processes, the exact Cr(III) content has not been reported. This gap in knowledge weakens this counter argument considerably. Ferrochrome exposures contain about 10-30% soluble chromium, presumably as Cr(VI), while stainless steel fumes have 0.005 - 4.3% water-soluble Cr(VI) (the top of the range refers to MMA/SS welding). It is obvious from this that these two groups of workers experience mixed exposure.

We conclude that the current integrated evidence does not permit the division of chromium compounds into carcinogenic and non-carcinogenic. It is clear that the sparingly soluble chromates (hexavalent) have recognized carcinogenic potency, while incriminating evidence is mounting for soluble chromates (hexavalent). Although the projected risk associated with exposure to trivalent chromium does not seem to be of the same order of magnitude as hexavalent chromium, it cannot be concluded that Cr(III) compounds are devoid of carcinogenic potential. Since not much is known about exposures to chromium metal, no conclusions are possible.

The issue of chromium carcinogenicity is more complex than just valence state and water solubility. Other characteristics that are likely of importance are the size distribution and other factors which influence the deposition, clearance, retention and absorption of the inhaled particles and mists (e.g., chemical composition, crystallinity, phagocytic index, chemical transformation and body fluid dissolution rates). These factors will also determine the biological half-life and the intracellular bioavailability of the inhaled materials, and presumably therefore, also their carcinogenicity.

### 4.5.2 Dose-Response Relationships

Throughout the discussion of the epidemiologic evidence in Sections 4.4 and 4.5.1, the available exposure data was reported and integrated into our discussions. However, epidemiological studies have great difficulty in considering these factors. As Langård (1983) has pointed out, even when atmospheric concentrations of substance(s) in question are cited in these studies, usually measurements have only been carried out during a short time period at the end of the observation period (e.g., Machle and Gregorius, 1948; Mancuso and Hueper, 1951; Mancuso, 1951; Gafafer, 1953; Langård and Norseth, 1975; Langard *et al*., 1980; and Axelsson *et al*., 1980). Such measurements do not correspond to the period of exposure which is the focus of the epidemiological study, nor do they provide information about average exposure or changes in exposure over time. Consequently, the information is not suitable for attempting to relate quantitatively exposure and risk, and does not allow extrapolation to 'safe' levels, nor the determination of maximum allowable exposures in the occupational setting (Hayes, 1980, 1982; Langård, 1983). Moreover, ideally the dose should be defined as the amount of a specific carcinogenic substance taken up during a given time. This requires the identification of the carcinogen. To complicate the matter further, the numerical assessment of risk is also dependent on a variety of methodological variables related to the epidemiologic method adopted (see framework for the interpretation of epidemiologic data in Appendix III). It is concluded, therefore, that the dose-response data available are inadequate to allow a quantitative risk assessment, which of course precludes an evaluation of risk at low levels of exposure. However, the epidemiologic studies reviewed do provide some indirect evidence concerning dose-response relationships. Using the duration of employment as an indicator of exposure, authors were frequently able to demonstrate that workers employed longer had a higher risk of respiratory cancer. The associated average latency period estimated from the interval between initial employment to the date of cancer diagnosis is estimated to be 18 ± 5 y (Enterline, 1974; Hayes, 1982).

Figure 4.1 Mutagenicity and clastogenicity of soluble hexavalent chromium compounds (includes both moderately and highly water-soluble chromates). See footnotes (a) and (b) to Table 4.1 for details of the assay systems employed. Numbers identify the original reference to which the data refer (see appended index on page 4-54). An individual summary for most studies is provided in Appendix I.

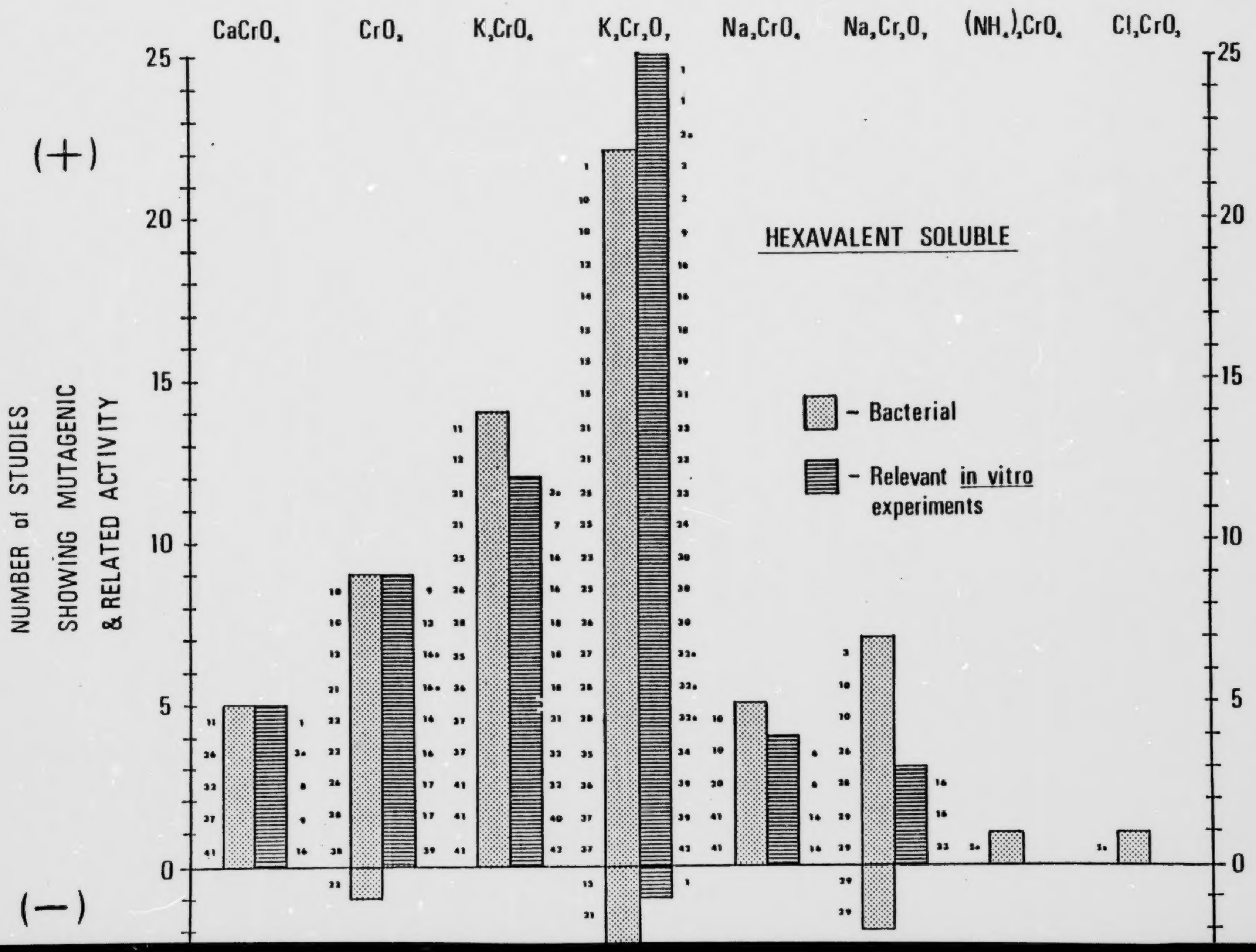

CaCrO$_4$
CrO$_3$
K$_2$CrO$_4$
K$_2$Cr$_2$O$_7$
Na$_2$CrO$_4$
Na$_2$Cr$_2$O$_7$
(NH$_4$)$_2$CrO$_4$
Cl$_2$CrO$_2$
NUMBER of STUDIES SHOWING MUTAGENIC & RELATED ACTIVITY
(+)
(−)
25
20
15
10
5
0
HEXAVALENT SOLUBLE
▨ – Bacterial
▤ – Relevant in vitro experiments

Figure 4.2 Mutagenicity and clastogenicity of insoluble hexavalent chromium compounds (includes water-insoluble to sparingly water-soluble chromates). See legend to Fig. 4.1 for experimental details and sources.

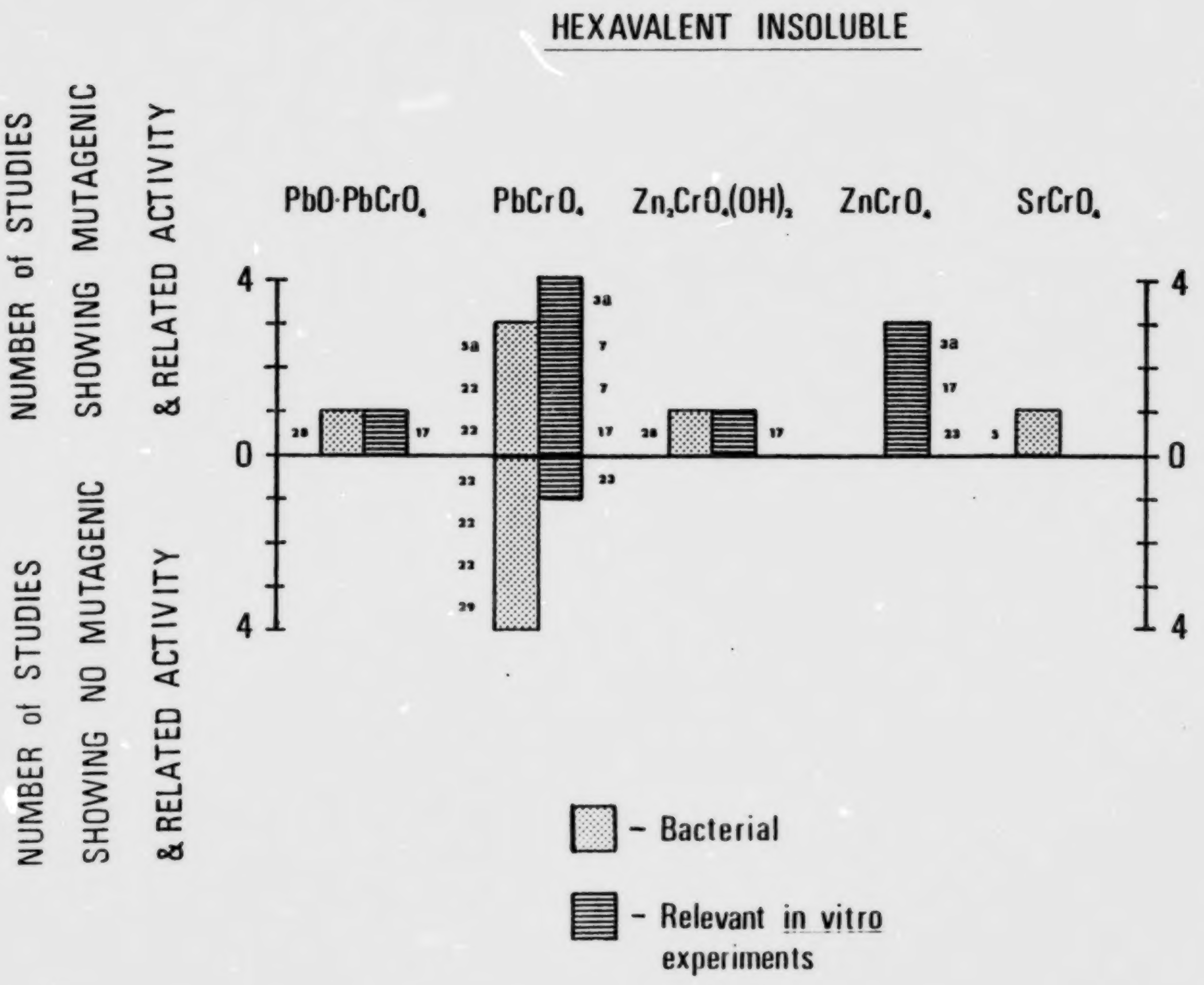

HEXAVALENT INSOLUBLE
NUMBER of STUDIES SHOWING MUTAGENIC & RELATED ACTIVITY
NUMBER of STUDIES SHOWING NO MUTAGENIC & RELATED ACTIVITY
PbO·PbCrO₄
PbCrO₄
Zn₂CrO₄(OH)₂
ZnCrO₄
SrCrO₄
4
0
4
28
17
38
22
22
38
7
7
17
22
22
22
29
23
28
17
38
17
23
5
- Bacterial
- Relevant in vitro experiments

Figure 4.3 Mutagenicity and clastogenicity of water-soluble trivalent chromium compounds. See legend to Fig. 4.1 for experimental details and sources.

## TRIVALENT SOLUBLE

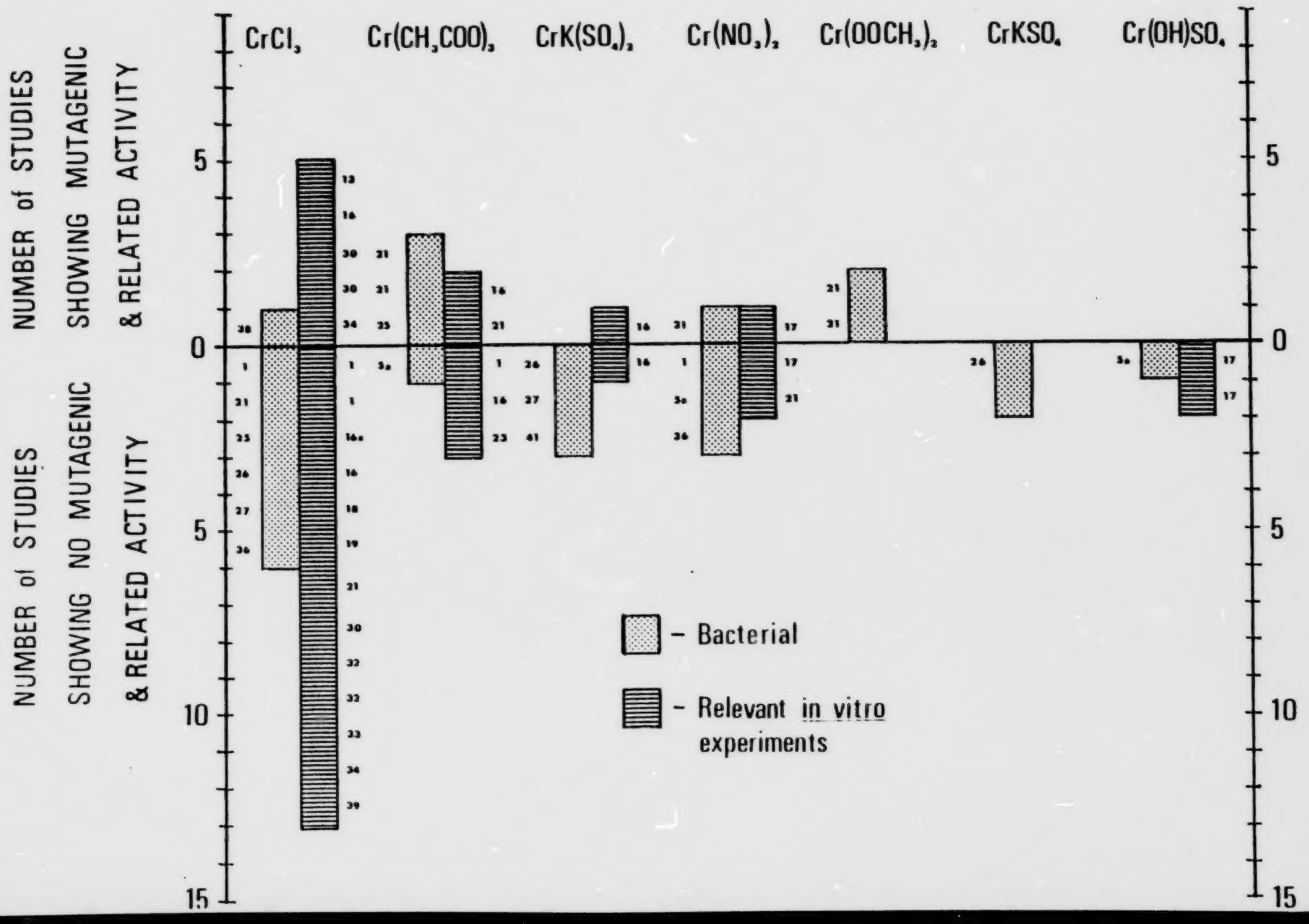
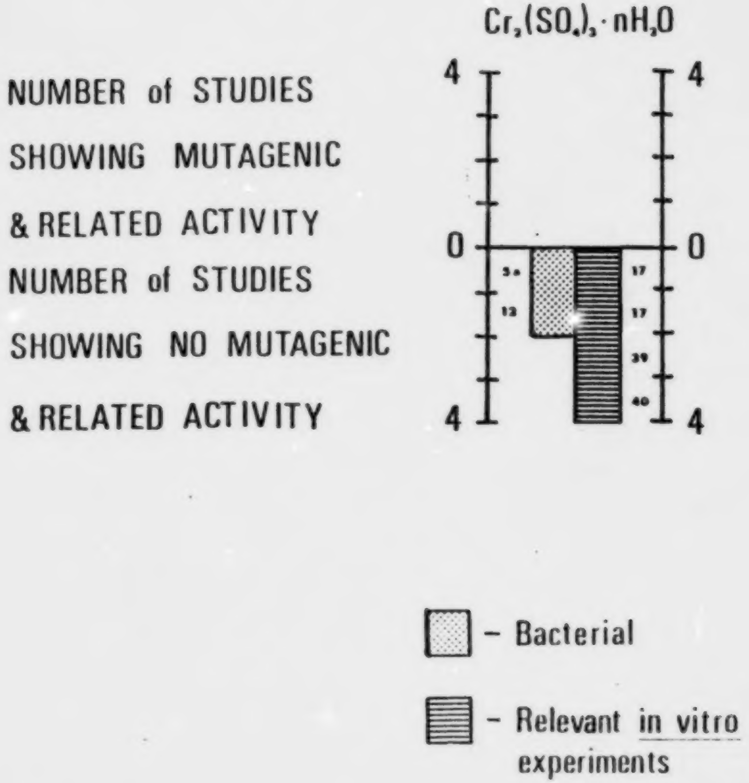


Figure 4.4 Mutagenicity and clastogenicity of trivalent chromium sulphates of unspecified solubilities. See legend to Fig. 4.1 for experimental details and sources.

## CHROMIUM (III) SULPHATES



## INDEX TO REFERENCES IN FIGURES 4.1 - 4.4

1. Bianchi et al., 1982
2a. Bigaliev et al., 1977
2. Bonatti et al., 1976
3a. Casto et al., 1979
3. De Flora et al., 1979

5a. De Flora, 1981
5. De Flora, 1982
6. Di Paolo and Casto, 1979
7. Douglas et al., 1980
8. Fradkin et al., 1975
9. Gomez-Arroyo et al., 1981
10. Gentil et al., 1981
11. Green et al., 1976
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16a. Koshi, 1979
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20. Mohn and Ellenberger, 1977
21. Nakamuro et al., 1978
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24. Nishioka, 1975
25. Nishioka, 1975
26. Petrilli and De Flora, 1977
27. Petrilli and De Flora, 1978a
28. Petrilli and De Flora, 1978b
29. Petrilli and De Flora, 1982
30. Raffetto et al., 1977
31. Rainaldi et al., 1980
32a. Rainaldi et al., 1982
32b. Rivedal and Sanner, 1981
33. Sarto et al., 1980
34. Stella et al., 1982
35. Tamaro et al., 1975
36. Tamaro et al., 1975
37. Tindall et al., 1978
38. Tkeshelashvili et al., 1980
39. Tsuda and Kato, 1977
40. Umeda and Nishmura, 1979
41. Venitt and Levy, 1974
42. Whiting et al., 1979

Figure 4.5 Carcinogenicity of calcium chromate in animals. Numbers identify the original reference to which the data refer (see appended index on page 4-73). An individual summary of each study is provided in Appendix II.

# CALCIUM

(Moderately Soluble)

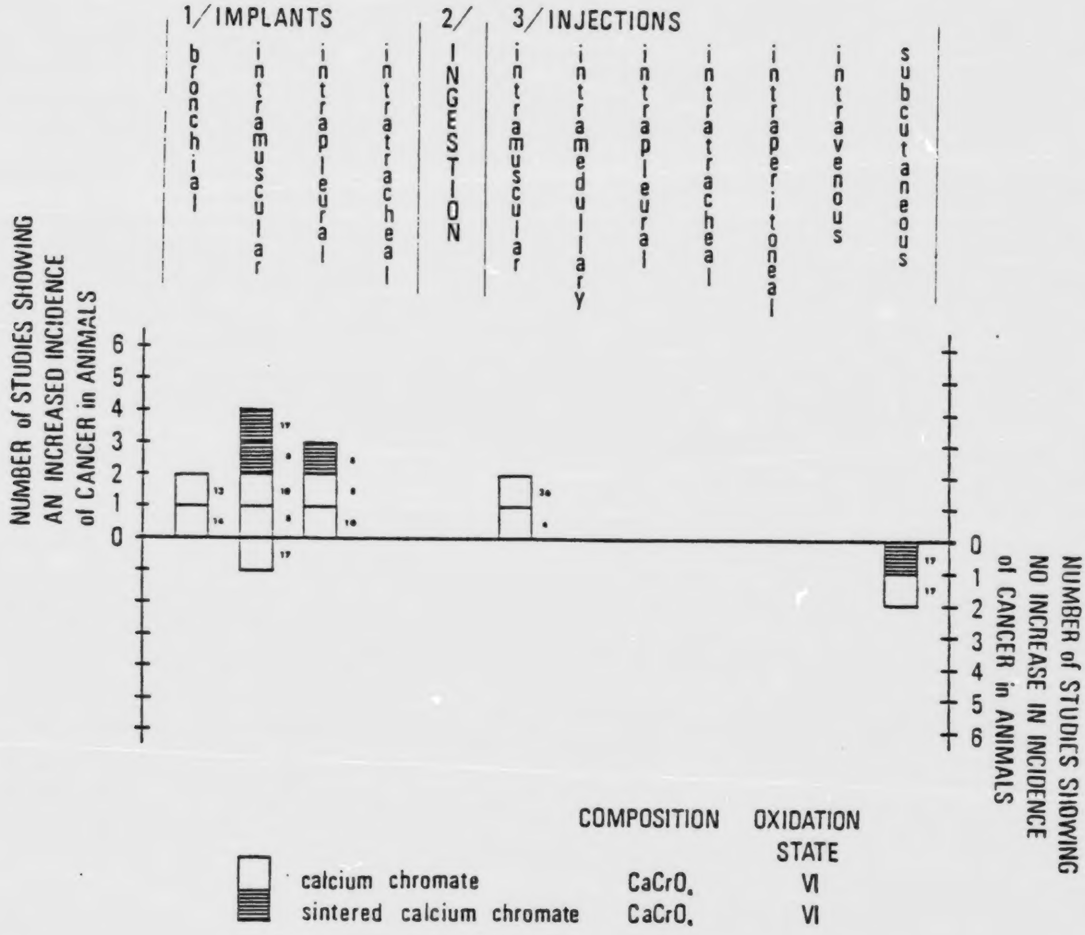


Figure 4.6 Carcinogenicity of lead chromate in animals. See legend to Fig. 4.5 for explanatory notes.

# LEAD

## (Sparingly Soluble)

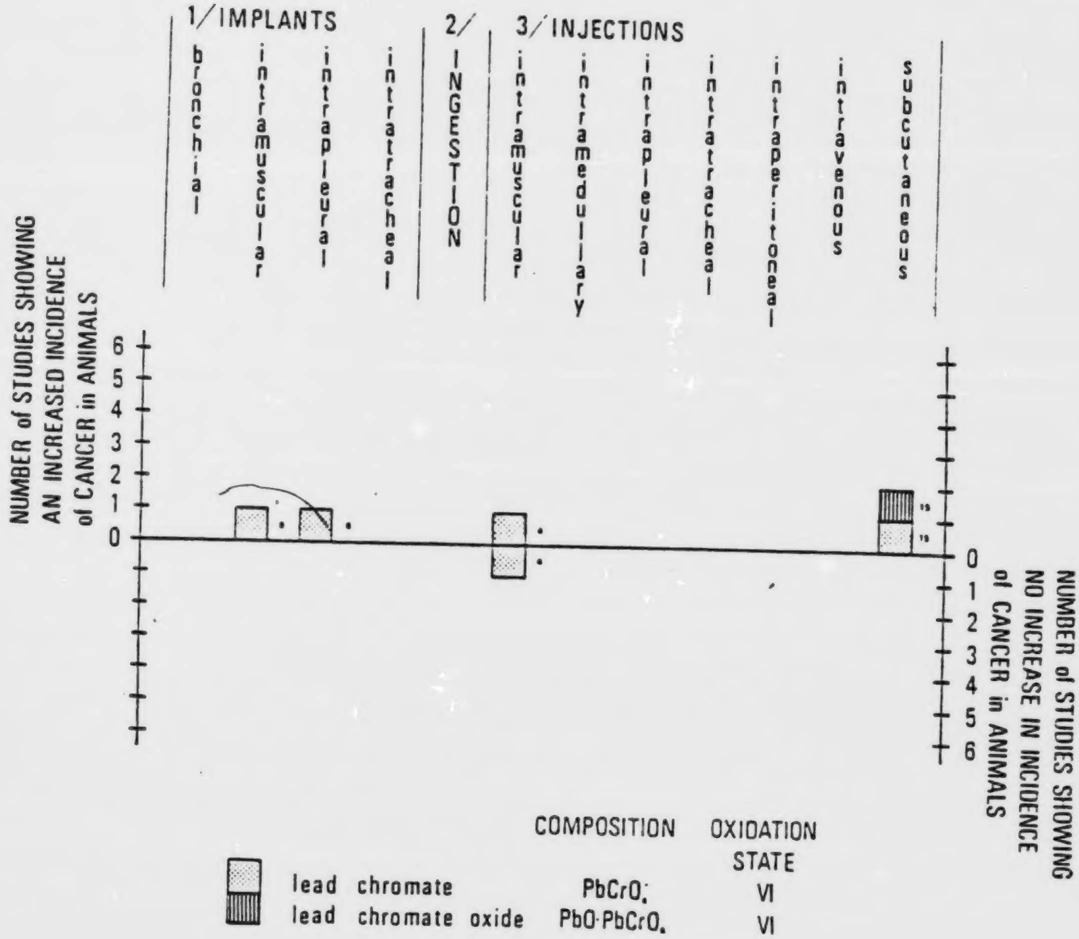


Figure 4.7 Carcinogenicity of chromium (III), strontium and zinc chromates in animals. See legend to Fig. 4.5 for explanatory notes.

## ZINC, STRONTIUM & CHROMIUM (III)

### (Sparingly Soluble)

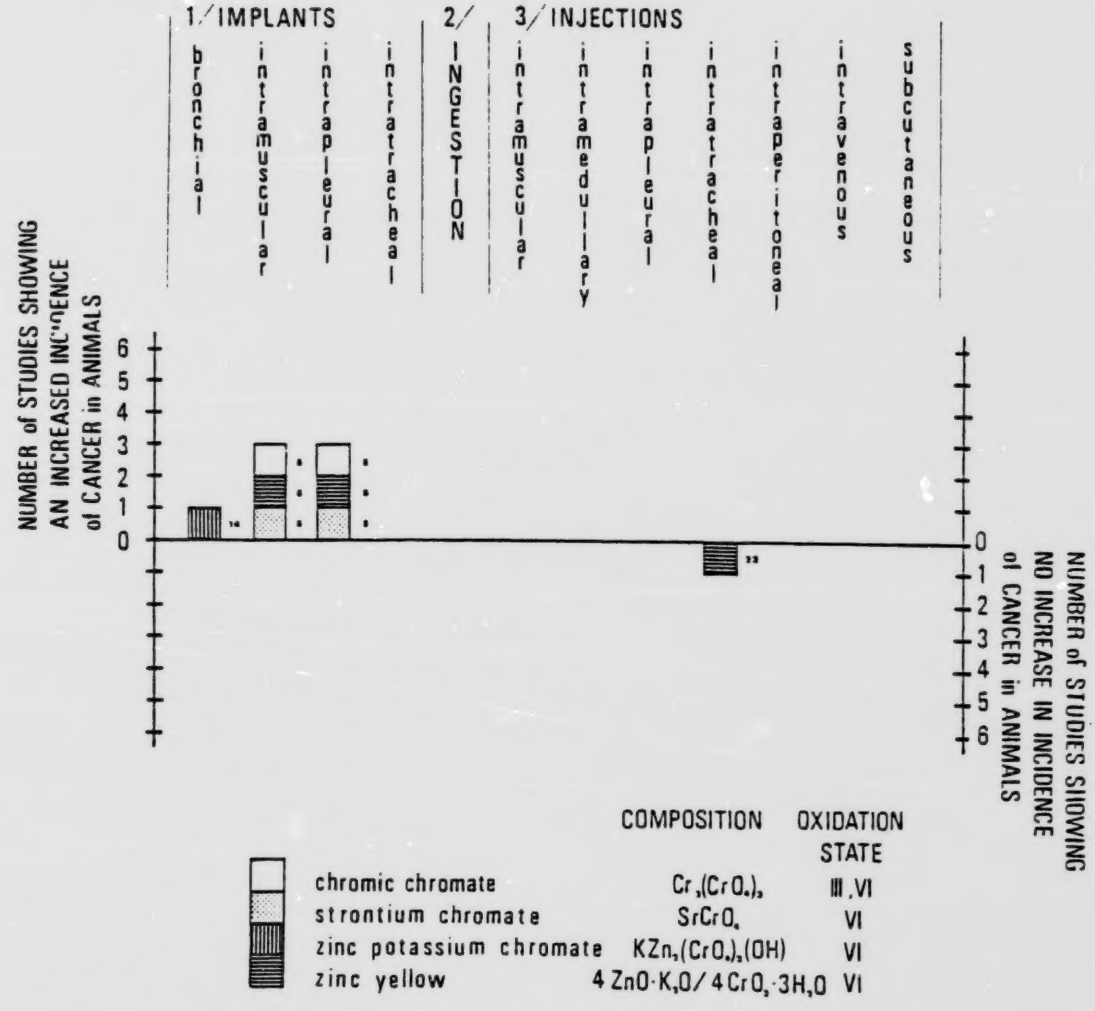


Figure 4.8 Carcinogenicity of water-insoluble chromium compounds in animals. See legend to Fig. 4.5 for explanatory notes.

# INSOLUBLE

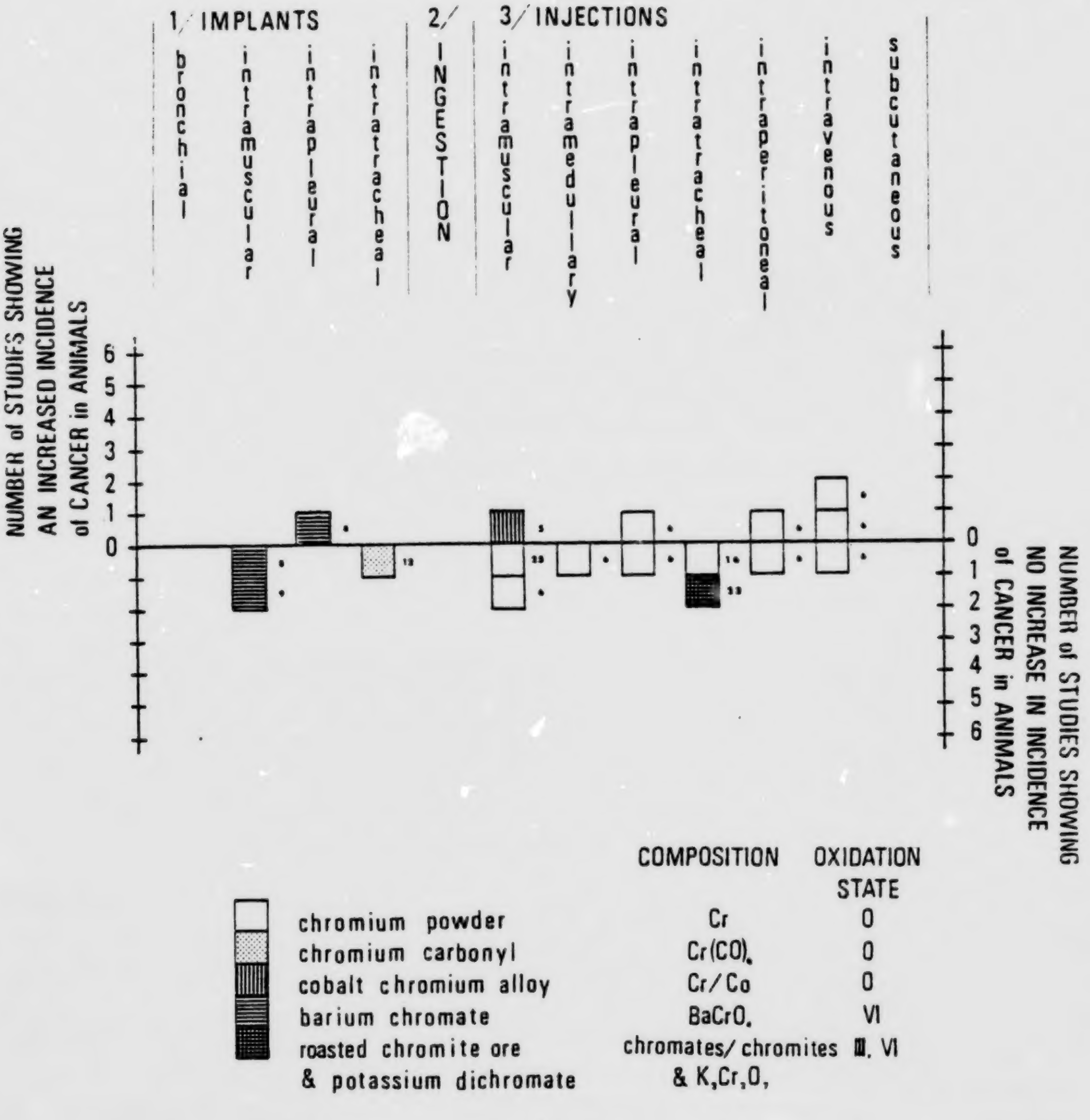

1/ IMPLANTS
2/
3/ INJECTIONS
bronchial
intramuscular
intrapleural
intratracheal
INGESTION
intramuscular
intramedullary
intrapleural
intratracheal
intraperitoneal
intravenous
subcutaneous
NUMBER of STUDIES SHOWING AN INCREASED INCIDENCE of CANCER in ANIMALS
6
5
4
3
2
1
0
NUMBER of STUDIES SHOWING NO INCREASE IN INCIDENCE of CANCER in ANIMALS
0
1
2
3
4
5
6
12
5
23
14
33
COMPOSITION
OXIDATION STATE
chromium powder
Cr
0
chromium carbonyl
$Cr(CO)_6$
0
cobalt chromium alloy
Cr/Co
0
barium chromate
$BaCrO_4$
VI
roasted chromite ore & potassium dichromate
chromates/chromites & $K_2Cr_2O_7$
III, VI

Figure 4.9 Carcinogenicity of chromium oxides in animals. See legend to Fig. 4.5 for explanatory notes.

# OXIDES

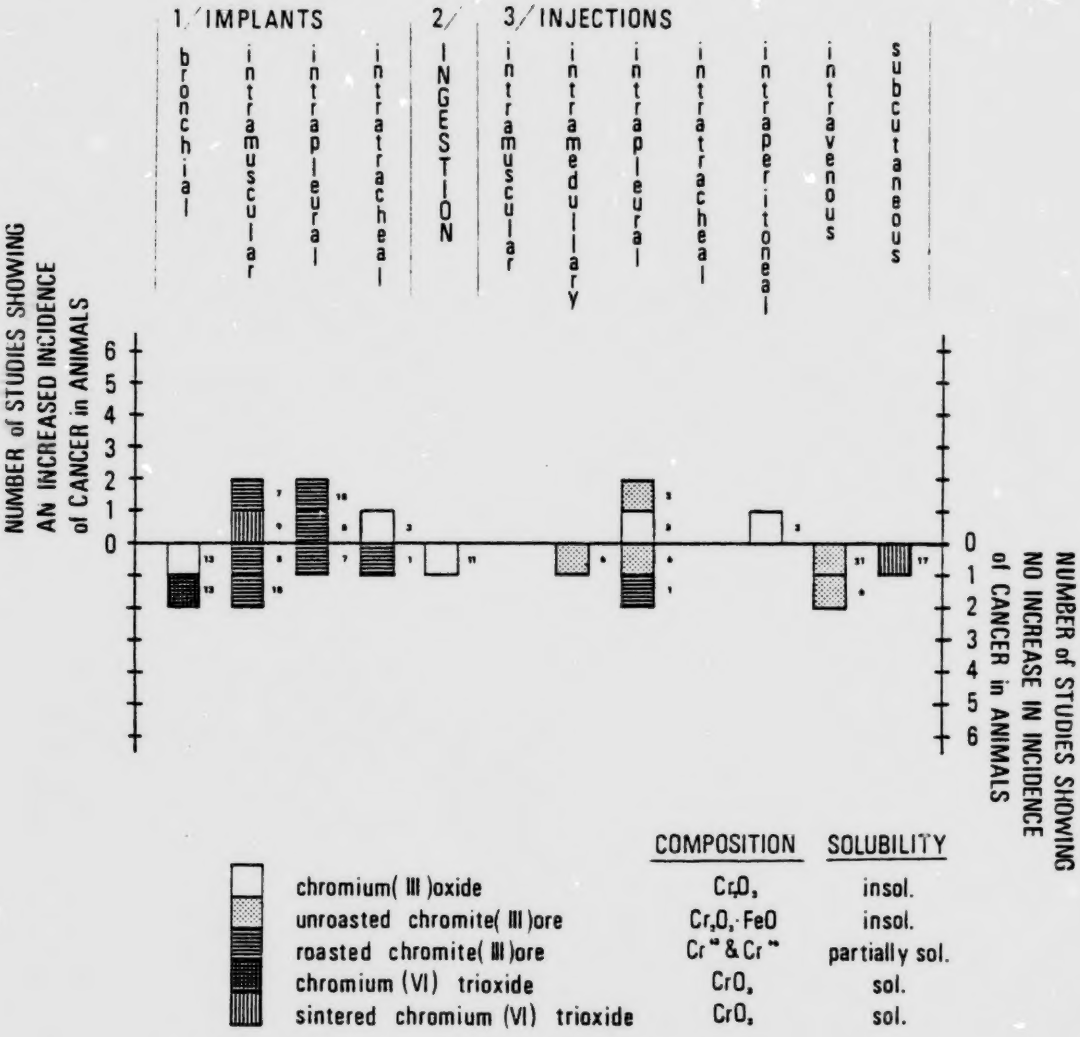
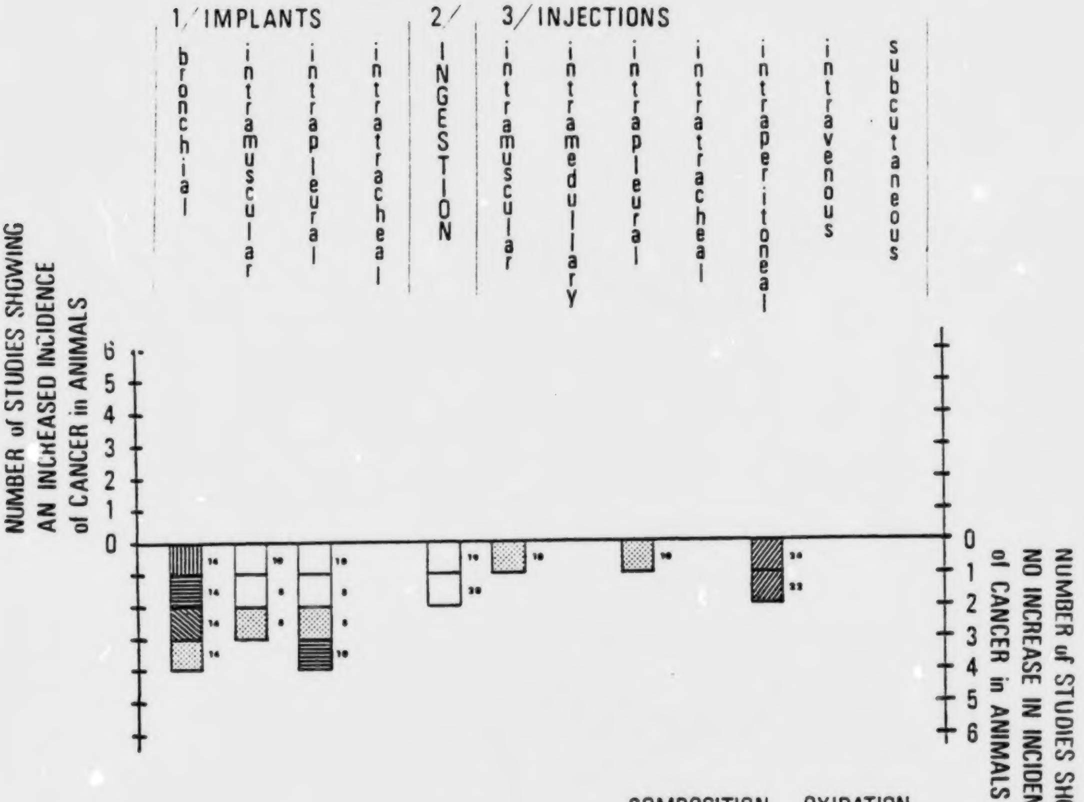

1/ IMPLANTS
bronchial
intramuscular
intrapleural
intratracheal
2/ INGESTION
3/ INJECTIONS
intramuscular
intramedullary
intrapleural
intratracheal
intraperitoneal
intravenous
subcutaneous
NUMBER of STUDIES SHOWING AN INCREASED INCIDENCE of CANCER in ANIMALS
NUMBER of STUDIES SHOWING NO INCREASE IN INCIDENCE of CANCER in ANIMALS
0
1
2
3
4
5
6
COMPOSITION
SOLUBILITY
chromium(III)oxide
Cr₂O₃
insol.
unroasted chromite(III)ore
Cr₂O₃·FeO
insol.
roasted chromite(III)ore
Cr³⁺ & Cr⁶⁺
partially sol.
chromium (VI) trioxide
CrO₃
sol.
sintered chromium (VI) trioxide
CrO₃
sol.

Figure 4.10 Carcinogenicity of water-soluble chromium(III) and chromium(VI) compounds in animals (includes the moderately-soluble potassium dichromate). See legend to Fig. 4.5 for explanatory notes.

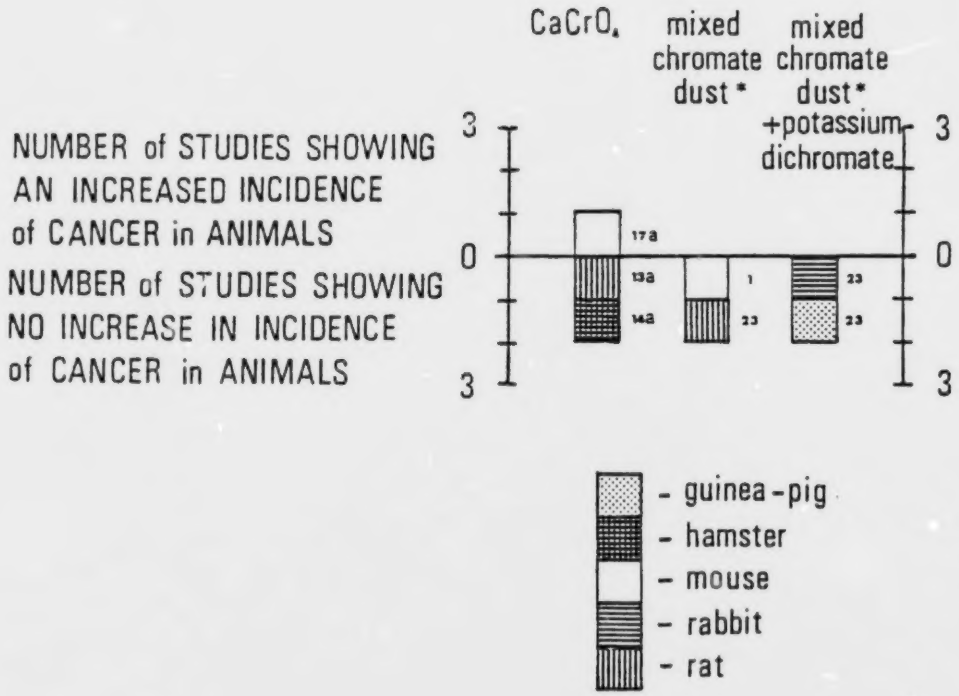
## SOLUBLE



| | | COMPOSITION | OXIDATION STATE |
|---|---|---|---|
| □ | chromium acetate | $Cr(CH_3COO)_3$ | III |
| ▨ | chromium sulphate | $Cr_2(SO_4)_3 \cdot nH_2O$ | III |
| ▧ | sodium chromate | $Na_2CrO_4$ | VI |
| ▥ | potassium chromate | $K_2CrO_4$ | VI |
| ▤ | potassium dichromate | $K_2Cr_2O_7$ | VI |
| ░ | sodium dichromate | $Na_2Cr_2O_7$ | VI |

Figure 4.11 Carcinogenicity in animals of selected chromium compounds administered by inhalation. See legend to Fig. 4.5 for explanatory notes.

## INHALATION



*mixed chromate dust: roasted chromite ore (III + VI)

Figure 4.12 Percentage incidence of administration-site tumours *versus* dose for calcium chromates. The data correspond to the results of the studies summarized in Fig. 4.5 and refer to experiments with rats or mice (given in parentheses).

| | | |
|---|---|---|
| ● | $CaCrO_4$ | 1. intramuscular injection or implantation |
| ○ | $CaCrO_4$, sintered | 2. intrapleural implantation |

a. Cumulative dose, rather than a single application.

# CALCIUM CHROMATE

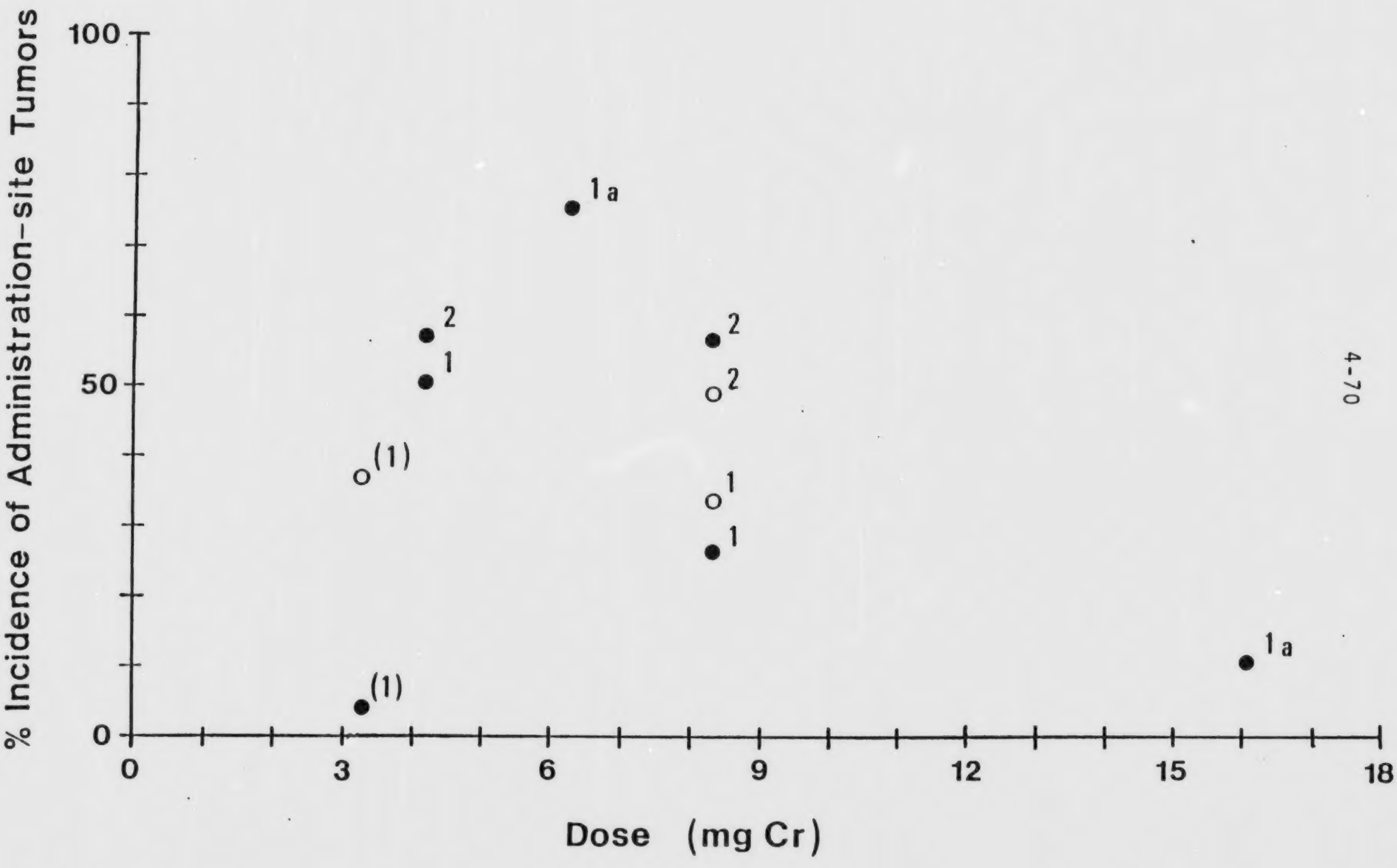


Figure 4.13 Percentage incidence of administration-site tumours *versus* dose for metal chromates (of chromium, lead, strontium and zinc). The data correspond to the results of the studies summarized in Figs. 4.6 and 4.7, and refer to experiments with rats or mice (given in parentheses).

● $PbCrO_4$

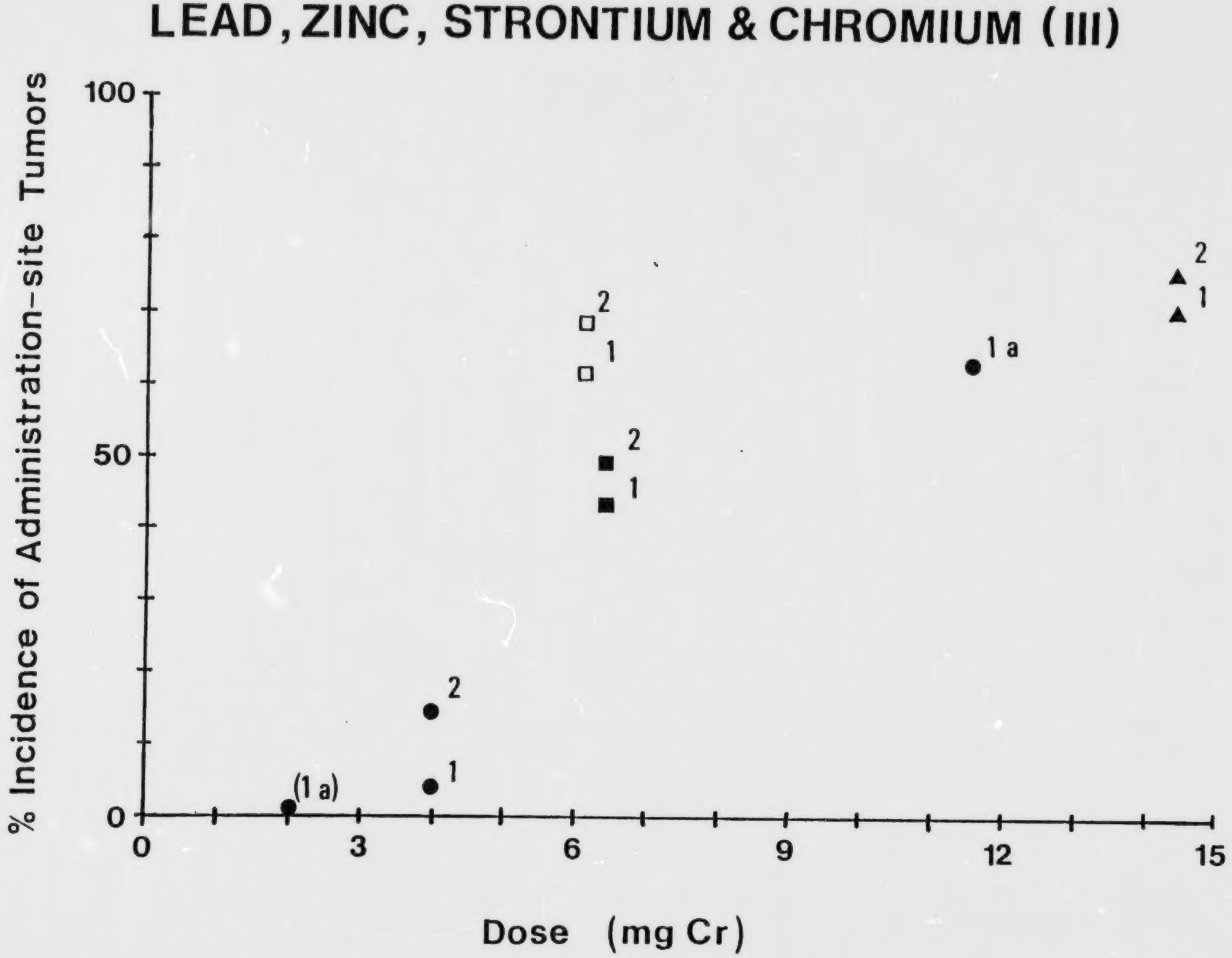
■ $SrCrO_4$

▲ $Cr_2(CrO_4)_3$

□ zinc yellow
($K_2CrO_4 \cdot 3ZnCrO_4 \cdot Zn(OH)_2 \cdot 2H_2O$)

1. intramuscular injection or implantation
2. intrapleural implantation

a. Cumulative dose rather than a single application.


LEAD, ZINC, STRONTIUM & CHROMIUM (III)
% Incidence of Administration-site Tumors
100
50
0
0
3
6
9
12
15
Dose (mg Cr)
(1 a)
1
2
1
2
1
2
1 a
2
1

## INDEX TO REFERENCES IN FIGURES 4.5 - 4.13

1. Baetjer et al., 1959a
2. Davis, 1972
3. Dvizhkov and Fedorova, 1967
4. Furst et al., 1976
5. Heath et al., 1971
6. Hueper, 1955
7. Hueper, 1958
8. Hueper, 1961
9. Hueper and Payne, 1959
10. Hueper and Payne, 1962
11. Ivankovic and Preussmann, 1975
12. Lane and Mass, 1977
13. Laskin et al., 1970
13a. Laskin, 1972
14. Levy and Venitt, 1975
14a. Laskin et al., 1972?
15. Maltoni 1974, 1976
16. Mukubo, 1978
17. Payne, 1960a
17a. Nettesheim et al., 1971
18. Payne, 1960b
19. Schroeder et al., 1964
20. Schroeder et al., 1965
21. Shimkin and Leiter, 1940
22. Shimkin et al., 1978
23. Steffee and Baetjer, 1965
24. Stoner et al., 1976
25. Sunderman *et al*., 1974
26. Roe and Carter, 1969

## Table 4.1

## A Summary of *In Vitro* Studies Relevant to Chromium Mutagenicity and Clastogenicity

### Bacterial Mutagenicity[a,c]

Hexavalent

| | No. of Studies | | |
|---|---|---|---|
| | + | - | total |
| sol. | 54 | 6 | 60 |
| insol. | 6 | 4 | 10 |
| total | 60 | 10 | 70 |

Trivalent

| | No. of Studies | | |
|---|---|---|---|
| | + | - | total |
| sol. | 7 | 16 | 23 |
| insol. | 0 | 2 | 2 |
| total | 7 | 18 | 25 |

### Other Relevant *In Vitro* Experiments[b,c]

Hexavalent

| | No. of Studies | | |
|---|---|---|---|
| | + | - | total |
| sol. | 58 | 1 | 59 |
| insol. | 9 | 1 | 10 |
| total | 67 | 2 | 69 |

Trivalent

| | No. of Studies | | |
|---|---|---|---|
| | + | - | total |
| sol. | 9 | 21 | 30 |
| insol. | 0 | 4 | 4 |
| total | 9 | 25 | 34 |

[a] Systems studied include *Escherichia coli* and *Salmonella typhimurium* for mutational changes, and *Bacillus subtilis* and *E. coli* for the study of DNA damage in repair-deficient strains.

[b] Relevant *in vitro* studies include experiments testing for chromosomal aberrations, sister chromatid exchange, cell transformation, gene mutations and stimulation of DNA-repair synthesis in mammalian cell culture systems.

[c] Soluble includes moderately and highly water soluble; insoluble includes sparingly water soluble and water insoluble (see Section 3.2.2.2).

## Table 4.2A

The Location of Chromate Manufacturing Plants From Which Vital Statistics Were Obtained in Epidemiological Studies [a,c]

| Location of Plant | Machle and Gregorius, 1948 | Brinton et al., 1952 | Gafafer, 1953 | Taylor, 1966 | Enterline, 1974 | Mancuso and Hueper, 1951 | Mancuso, 1975 | Baetjer, 1950a | Hayes et al., 1979 | Hill and Ferguson, 1979 | Bidstrup, 1951 | Bidstrup and Case, 1956 | Alderson et al., 1981 | Ohsaki et al., 1978 | Satoh et al., 1981 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Painesville, OH | + | + | + | + | + | + | + | - | - | - | - | - | - | - | - |
| Baltimore, MD | + | + | + | + | + | - | - | + | + | + | - | - | - | - | - |
| Jersey City, NJ (2 plants) | + | + | + | +[b] | +[b] | - | - | - | - | - | - | - | - | - | - |
| Newark, NJ | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| Kearny, NJ | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| Glens Falls, NY | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| Bolton, England | - | - | - | - | - | - | - | - | - | - | + | + | + | - | - |
| Eaglescliffe, UK | - | - | - | - | - | - | - | - | - | - | + | + | + | - | - |
| Rutherglen, Scotland | - | - | - | - | - | - | - | - | - | - | + | + | + | - | - |
| Hokkaido Island, Japan | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - |
| Tokyo, Japan | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + |

[a] + Participating chromate manufacturing plant - Non-participating chromate manufacturing plant.

[b] Only the larger of the 2 plants was studied.

[c] The available environmental data are reported in Table 4.2B.

## Table 4.2B Environmental Data for Chromate Manufacturing Plants (in mg Cr $m^{-3}$)

### A. Chromate Production in the U.S.A. and Great Britain.

| Plant | Great Britain | | | Painesville | | | E(Painesville) | C(Glens Falls) | D1 | A1 |
|---|---|---|---|---|---|---|---|---|---|---|
| Exposure to Cr | alkali insol | alkali sol | total | water insol | water sol | total | total | total | total | total |
| ore prep | | | | 1.33 | 0.03 | 1.36 | 0.005–0.70 | 0.15–1.40 | 0.40–2.30 | 0.03–0.50 |
| mixing of reactants | 2.14 | 0.005 | 2.15 | 1.20 | 0.10 | 1.30 | | | | |
| roasting | 0.17 | 0.03 | 0.20 | 0.39 | 0.23 | 0.62 | | | | |
| extraction of monochromate | 0.04 | 0.52 | 0.56 | 0.10 | 0.10 | 0.20 | | | | |
| neutralization | | | | 0.03 | 0.06 | 0.09 | | | | |
| formation of dichromate | 0.01 | 0.06 | 0.07 | 0.09 | 0.10 | 0.19 | | | | |
| separation of dichromate/liquor | | | | 0.13 | 0.27 | 0.40 | | | | |
| processing sodium sulfate | | | | 0.88 | 0.24 | 1.12 | | | | |
| concentration | 0.005 | 0.88 | 0.89 | | | | 0.07–0.14 | 0.01 | 1.09 | 0.01–0.10 |
| drying | | | | 0.30 | 0.18 | 0.48 | 0.13 | 0.02 | 0.17 | 0.10 |
| granulation | | | | | | | 0.06–0.07 | 0.005–0.37 | | 0.02–0.29 |
| packing | 0.009 | 0.47 | 0.48 | | | | 2.5–10.5 | | | 0.10 |
| general air | | | | | | | 0.002 | | 0.02 | 0.01–0.30 |

The reported values are the result of a survey performed in 1949–50 when 96 air samples from the 3 plants were taken and analyzed (Buckell and Harvey, 1951).

The environmental hygiene survey was conducted in early 1949 and the levels reported do not reflect the greater, though steadily decreasing, atmospheric chromium contamination of the plant during the time since it began operations in 1932. A total of 121 air samples were analyzed (Bourne and Yee, 1950).

Air sampling and analysis was conducted at some time during 1941–48 (Machle and Gregorius, 1948). It was reported that the magnitude of the chromium exposure had progressively lessened during the 15–20 years prior to 1948.

### B. Detailed Survey of the Six U.S.A. Plants (Gafafer, 1953) (1600 samples collected c 1950)

B1. Frequency distribution of air concentrations of total chromium for various plant operations.

| Functional group | Total number of samples | Percent of samples within indicated range (milligrams Cr per cubic meter of air) | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0.00<br>0.05 | 0.06<br>0.10 | 0.11<br>0.15 | 0.16<br>0.20 | 0.21<br>0.25 | 0.26<br>0.30 | 0.31<br>0.35 | 0.36<br>0.40 | 0.41<br>0.50 | 0.51<br>0.60 | 0.61<br>0.70 | 0.71<br>0.80 | 0.81<br>0.90 | 0.91<br>1.0 | 1.01<br>1.50 | 1.51<br>2.00 | 2.01<br>3.00 | over<br>3.00 |
| Ore processing | 28 | 14 | 7 | 18 | 4 | 0 | 7 | 3 | 0 | 4 | 0 | 4 | 0 | 4 | 0 | 14 | 11 | 7 | 3 |
| Residue processing | 16 | 6 | 6 | 13 | 6 | 6 | 12 | 13 | 7 | 19 | 0 | 0 | 6 | 0 | 0 | 6 | | | |
| Roasting | 32 | 6 | 13 | 13 | 16 | 3 | 9 | 6 | 0 | 13 | 4 | 6 | 3 | 0 | 0 | 3 | 3 | 0 | 3 |
| Leaching | 15 | 6 | 13 | 13 | 7 | 0 | 7 | 7 | 0 | 13 | 0 | 7 | 0 | 0 | 0 | 7 | 7 | 7 | 6 |
| Neutralizing and treating | 33 | 9 | 15 | 12 | 9 | 9 | 9 | 7 | 6 | 6 | 6 | 0 | 0 | 3 | 3 | 0 | 3 | 3 | |
| Concentrating and granulating | 10 | 30 | 10 | 20 | 30 | 0 | 0 | 10 | | | | | | | | | | | |
| Filtering, drying and packing | 15 | 0 | 0 | 27 | 7 | 13 | 7 | 7 | 0 | 7 | 13 | 7 | 0 | 0 | 0 | 6 | 0 | 0 | 6 |
| Other products | 30 | 27 | 13 | 17 | 13 | 4 | 4 | 0 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 3 | 0 | 3 | 10 |

B2. Frequency distribution of air concentrations of water soluble chromium (hexavalent) for various plant operations.

| Functional group | Total number of samples | Percent of samples within indicated range (milligrams Cr per cubic meter of air) | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0.00<br>0.05 | 0.06<br>0.10 | 0.11<br>0.15 | 0.16<br>0.20 | 0.21<br>0.25 | 0.26<br>0.30 | 0.31<br>0.35 | 0.36<br>0.40 | 0.41<br>0.50 | 0.51<br>0.60 | 0.61<br>0.70 | 0.71<br>0.80 | 0.81<br>0.90 | 0.91<br>1.0 | 1.01<br>1.50 | 1.51<br>2.00 | 2.01<br>3.00 | over<br>3.00 |
| Ore processing | 78 | 92 | 7 | 1 | | | | | | | | | | | | | | | |
| Residue processing | 90 | 51 | 18 | 10 | 3 | 6 | 2 | 4 | 4 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | | |
| Roasting | 116 | 53 | 14 | 9 | 4 | 8 | 3 | 2 | 2 | 2 | 0 | 1 | 1 | 1 | 1 | 2 | 0 | 2 | 1 |
| Leaching | 96 | 38 | 22 | 7 | 11 | 6 | 6 | 1 | 2 | 3 | 2 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | |
| Neutralizing and treating | 200 | 51 | 18 | 14 | 5 | 6 | 1 | 2 | 1 | 2 | 1 | 1 | ½ | 1 | ½ | | | | |
| Concentrating and granulating | 84 | 63 | 25 | 7 | 2 | 1 | 0 | 2 | | | | | | | | | | | |
| Filtering, drying and packing | 101 | 48 | 11 | 3 | 6 | 8 | 4 | 1 | 2 | 4 | 2 | 4 | 4 | 0 | 1 | 0 | 0 | 0 | 1 |
| Other products | 160 | 65 | 16 | 6 | 3 | 3 | 1 | 1 | 1 | ½ | 0 | ½ | 0 | ½ | 1 | 0 | 0 | 0 | ½ |

B3. Frequency distribution of air concentrations of acid soluble - water insoluble chromium (mostly trivalent, > 96%) for various plant operations.

| Functional group | Total number of samples | Percent of samples within indicated range (milligrams Cr per cubic meter of air) | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0.00<br>0.05 | 0.06<br>0.10 | 0.11<br>0.15 | 0.16<br>0.20 | 0.21<br>0.25 | 0.26<br>0.30 | 0.31<br>0.35 | 0.36<br>0.40 | 0.41<br>0.50 | 0.51<br>0.60 | 0.61<br>0.70 | 0.71<br>0.80 | 0.81<br>0.90 | 0.91<br>1.0 | 1.01<br>1.50 | 1.51<br>2.00 | 2.01<br>3.00 | over<br>3.00 |
| Ore processing | 28 | 54 | 18 | 11 | 10 | 0 | 4 | 0 | 3 | | | | | | | | | | |
| Residue processing | 16 | 25 | 38 | 7 | 12 | 0 | 6 | 0 | 0 | 6 | | | | | | | | | |
| Roasting | 32 | 69 | 13 | 3 | 9 | 0 | 3 | 0 | 0 | 3 | | | | | | | | | |
| Leaching | 21 | 62 | 0 | 0 | 14 | 14 | 0 | 0 | 0 | 10 | | | | | | | | | |
| Neutralizing and treating | 32 | 69 | 9 | 4 | 6 | 3 | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 3 | | | |
| Concentrating and granulating | 10 | 80 | 20 | | | | | | | | | | | | | | | | |
| Filtering, drying and packing | 15 | 67 | 7[1] | 7[1] | 0 | 7[1] | 0 | 6[1] | 0 | 0 | 0 | 0 | 6[1] | | | | | | |
| Other products | 30 | 53 | 7 | 10[1] | 6[1] | 3 | 0 | 0 | 0 | 0 | 6[1] | 0 | 0 | 0 | 0 | 3[1] | 3[1] | 4[1] | 7[1] |

[1] Primarily tanning compound; Cr as basic chromium sulfate.

B4. Frequency distribution of air concentrations of acid insoluble chromium (chromite ore) for various plant operations

| Functional group | Total number of samples | Percent of samples within indicated range (milligrams Cr per cubic meter of air) | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0.00<br>0.05 | 0.06<br>0.10 | 0.11<br>0.15 | 0.16<br>0.20 | 0.21<br>0.25 | 0.26<br>0.30 | 0.31<br>0.35 | 0.36<br>0.40 | 0.41<br>0.50 | 0.51<br>0.60 | 0.61<br>0.70 | 0.71<br>0.80 | 0.81<br>0.90 | 0.91<br>1.0 | 1.01<br>1.50 | 1.51<br>2.00 | 2.01<br>3.00 | over<br>3.00 |
| Ore processing | 28 | 25 | 11 | 7 | 4 | 0 | 4 | 0 | 3 | 0 | 7 | 0 | 0 | 3 | 0 | 18 | 7 | 7 | 4 |
| Residue processing | 16 | 50 | 25 | 13 | 0 | 0 | 0 | 0 | 12 | | | | | | | | | | |
| Roasting | 32 | 66 | 13 | 4 | 0 | 6 | 3 | 0 | 3 | 0 | 0 | 0 | 3 | 3 | | | | | |
| Leaching | 21 | 70 | 0 | 0 | 10 | 5 | 0 | 0 | 0 | 5 | 0 | 5 | 0 | 0 | 0 | 5 | | | |
| Neutralizing and treating | 33 | 76 | 15 | 3 | 3 | 3 | | | | | | | | | | | | | |
| Concentrating and granulating | 10 | 100 | | | | | | | | | | | | | | | | | |
| Filtering, drying and packing | 15 | 100 | | | | | | | | | | | | | | | | | |
| Other products | 31 | 97 | 3 | | | | | | | | | | | | | | | | |

Table 4.3

Epidemiological Studies of Cancer in Workers in Chromate-Producing Industries

| | | | | Respiratory Cancer[a] | | Other Cancers | | |
|---|---|---|---|---|---|---|---|---|
| Reference | Study Design | Definition of Cohort, Follow-up (FU) and Analysis | Comparison Population | No. of Cases | Estimated Relative Risk | Site | No. of Cases | Estimated Relative Risk |
| Machle & Gregorius, 1948[b] | Historical Prospective Cohort | Active workers employed for 4-17 years prior to 1948; mortality based on life insurance claims; excluded those who left the industry. | Oil-refinery workers, cancer mortality 1933-38 | 42<br>oral: 3 | 25* (ranged from 18-50 in 5 of the 6 plants)<br>5.4 | Digestive | 13 | 2* |
| Baetjer, 1950[a,b] | Case Control | Lung cancer U.S. chromium plant. | Random sample of hospital admissions | 11 | Zero in Controls* | Not Reported | | |
| Brinton, et al., 1952; Gafafer, 1953 | Proportional Mortality Study | Members of sick-benefit associations who died within one year of becoming disabled during 1940-48. | U.S. male population 1940-48.<br>- white males<br>- black males | 26<br>10<br>16 | 29*<br>14<br>80 | all other | 6 | 0.95 |
| Mancuso & Hueper, 1952[a] | Proportional Mortality Study | Workers employed 1 year or more 1931-49; mortality based on death certificates, included those who left. | Proportional mortality for county, 1937-47. | 6 | 15* | Not Reported | | |
| Mancuso, 1975 | Proportional Mortality Study | Workers employed for 1 year or more in 1931-37; FU until 1974. | None | 41 | | Not Reported | | |
| Taylor, 1966 | Historical Prospective Cohort | Workers employed three or more months between 1937-40 and have earnings reported to OASDI; mortality based on death certificate until Dec. 1960 and who had death claim filed with OASDI, included workers who left the industry. | Age-specific mortality rates for the U.S. civilian male population. | 71 | 8.5* | all other | 32 | 1.3 |
| Enterline, 1974 | Historical Prospective Cohort | As above - based on recalculation of data for the years 1941-60 originally reported by Taylor, 1966. | As above | 69 | 9.4 | Digestive | 16 | 1.5 |
| Hayes et al., 1979 | Historical Prospective Cohort | Workers employed 3 or more months hired 1945-74; 88% follow-up by mid 1977. | Baltimore City mortality rates. | 59 | 2* | all other | 86 | 1.02 |
| | | | | | | Digestive | 13 | 0.6 |
| | Case-control Analysis w.r.t. work history | Analysis for cohort employed 3 years or more exclusively in the new plant with improved hygiene standards, hired 1950-59. | Internal comparison by worksite | 3 | 4* | | | |
| Bidstrup & Case, 1956[a] | Historical Prospective Cohort | Numbers employed in 1949 derived from X-ray survey; mortality based on death certificate entry to 1955; workers who left the industry (30%) were not followed. | Males from England & Wales 1951-53 | 12 | 3.6* | all other | 9 | 1.1 |
| Alderson et al., 1981 | Historical Prospective Cohort | Workers employed at least 1 year between 1948-77. | National mortality rates for England, Wales & Scotland | 116 (lung)<br>2 (nasal) | 2.4*<br>7.1 | all other | 80 | 1.2 |
| Satoh et al., 1981 | Historical Prospective Cohort | Workers employed for ≥ 1 year between 1918-75; FU until 1978. | Japanese males 1950-78. | 25 | 9.2 | all other | 24 | 0.8 |
| | | | | | | sinus (5), nasal (1) | | |
| Ohsaki et al., 1978 | Case Series | Cases diagnosed during 1972-76 and found in death certificates; insufficient detail.<br>1982 update (Tsuneta, 1982) | Japanese population 1975 | 14<br>24 | 50<br>16 | Not Reported | | |

* Statistical significance with $p < 0.05$

[a] Here and in subsequent Tables, respiratory cancer usually includes that of the trachea, bronchus, lung and larynx (ICD-161/2). Laryngeal cancer (ICD-161) is also reported separately if statistically significant on its own. Nasal cancer (ICD-160) statistics are shown separately.

[b] Environmental data for this study is summarized in Table 4.2.

## Table 4.4

## Summary of Epidemiological Studies of Cancer in Workers Employed in the Chromate Pigment Industry

| | | | | Respiratory Cancer | | Other Cancers | | | |
|---|---|---|---|---|---|---|---|---|---|
| Reference | Study Design | Definition of Cohort, Follow-up (FU) and Analysis | Comparison Population | No. of Cases | Estimate of Relative Risk | Site | No. of Cases | Estimate of Relative Risk | Exposure Data |
| Langård & Norseth, 1975 | Historical Prospective Cohort | 24 Workers with more than 3 years employment in a Norwegian chromate pigment plant; FU Norwegian Cancer Registry 1951-1972. | Cancer incidence rates for Norwegian males 1955-1971. | 3 | 38 | G.I.<br>nasal | 1[a]<br>1[a] | n/a<br>n/a | yes[e] |
| American Dry Color Manufacturers, 1976 (EEH, 1976) | Historial Prospective Cohort | Workers hired before 1960, employed 10 years or more; mortality based upon death certification as reported to OASDI. | Cancer mortality rates for U.S. males and for counties where plants were located. | | | | | | no[b] |
| | | - exposed to lead chromate only (plants 1 & 2) | | 2 | 2.4 | G.I. | 0 | n/a | |
| | | - exposed to lead and zinc chromates (plant 3) (but see 1983 report) | | 6 | 2.4* | stomach | 5 | 7.8 | |
| Davies, 1978, 1979, 1984a | Historical Prospective Cohort | Males initially employed ≥ 1 year prior to 1967 at 3 chromate pigment plants; deaths reported to the N.H.S. cancer registry to Dec. 31, 1981 | Quinary quinquennial national death rates covering 1931-5 onwards; not adjusted for regional or social class factors | | | | | | no[c] |
| | | - exposed to lead chromate only | | 7 | 1.1 | | | | |
| | | - zinc and lead chromates | | | | | | | |
| | | - high and medium exposure[c] | | 32 | 2.7* | Other resp. | 3 | 2.7 | |
| | | - low exposure[c] | | 7 | 1.8 | | | | |
| Frentzel-Beyme & Claude, 1980 | Historical Prospective Cohort | Workers in 7 factories in 4 countries; FU difficulties, insufficient detail. | Not reported | Increased risk, details not reported. | | Not reported | | | no |
| Sheffet et al., 1982 | Historical Prospective Cohort | Males employed for greater than 1 month during 1940-1969; 324 of 1946 lost to FU. | Cancer mortality rates for U.S. males adjusted for race. | Various expected rates given according to assumptions about those lost to FU, rel. risk ranging from 1.3-1.6*; | | stomach<br>pancreas | ranging 1.4-2.0<br>ranging 1.5-1.7 | | yes[d] |
| | Proportional Mortality Study | | | ranging from 1.5-2.0* | | stomach<br>pancreas | ranging 1.6-2.8*<br>ranging 1.6-2.2 | | |
| Langård & Vigander, 1983 | Historical Prospective Cohort | FU of cohort in study by Langard and Norseth, 1975 | | 6 | 44 | G.I.<br>nasal | 4[a]<br>1[a] | n/a<br>n/a | yes[e] |
| American Dry Color Manufacturers, 1983 (Cooper, 1983) | Historial Prospective Cohort | 6 months exposure prior to Jan.1975: | Cancer mortality rates for U.S. white males | | | | | | yes[b] |
| | | -plant 1 (lead chromate) | | 4 | 1.6 | stomach | 0 | n/a | |
| | | -plant 2 (mixed:Pb,Zn,Sr,Ba) | | 2 | 2.0 | stomach | 0 | n/a | |
| | | -plant 3 (mixed:Pb & Zn) | | 9 | 2.2* | stomach | 5 | 7.9* | |

n/a Not available.

* Statistical significance at $p < 0.05$.

[a] Those deaths refer to the cohort of all men who were ever employed at the pigment plants between 1948 and 1972, which numbered 133.

[b] Reported in the 1983 report as follows:
Lead levels of 11.0 - 51.6 mgPb/m$^3$ in 1957 in one plant. In a second plant the ranges and averages for lead were 0.01 - 10 mg/m$^3$ and 0.38 mgPb/m$^3$, respectively, in 1965-68; for 1968-72, 0.05 - 24.3 and 2.47 mgPb/m$^3$, respectively. Most ratios of Pb:Cr were between 4:1 and 6:1 in 1975.

[c] Exposure categories were defined as follows:
high - in "dry" departments where pigments were ground, blended and packaged;
medium - in "wet" departments where precipitates were washed, pressed and stove-dried;
low - most lab jobs, boiler stoking, painting, bricklaying.

[d] Exposure estimates during the study period, based on air samples collected in later years, were as follows (in mgCr/m$^3$)
high - >2
medium - 0.5-2
low - <0.1
The proportion of $PbCrO_4$ to $ZnCrO_4$ was approximately 9 to 1 in most areas of the plant.

[e] Exposure to Airborne Cr (in mg/m$^3$) in 3 Chromate Pigment Plants in Norway (Langård & Norseth, 1975)

| Operation | Plant A | Plant B | Plant C |
|---|---|---|---|
| Sackfilling | 0.43 | 1.35 | 0.08 |
| Mixing raw materials | 0.35 | 0.33 | 0.01 |
| Foreman | 0.19 | 0.04 | |

The hygiene survey was conducted circa 1972 with portable personal samplers, where N = 4 or 5 for each job class. Past exposures were at the same level as at the time of the survey, as ascertained by interviews with older employees. Plant C was constructed in 1972.

Table 4.5

Summary of Epidemiological Studies of Cancer in Workers Employed in the Ferrochromium Industry

| Reference | Study Design | Definition of Cohort Follow-up (FU) and Analysis | Comparison Population | Respiratory Cancer | | Other Cancers | | | Exposure Data |
|---|---|---|---|---|---|---|---|---|---|
| | | | | No. of Cases | Estimate of Relative Risk | Site | No. of Cases | Estimate of Relative Risk | |
| Pokrovskaya & Shabynina, 1973 | Historical Prospective Cohort | Workers employed in a Russian chromium ferroalloy smelting plant between 1955 and 1969; FU of death certificates from city archives; results given for males age 50-59. | Cancer mortality rates for the city in which the plant was located. | n/a | 3.2 | stomach<br>esophagus<br>all sites | n/a<br>n/a<br>n/a | 6.6*<br>2.0*<br>3.3* | yes |
| Langård et al., 1980 | Historical Prospective Cohort | Males employed for at least 1 year during 1928 - 1959 in a Norwegian ferroalloy plant; FU of deaths reported during 1953 - 1977. | Cancer mortality rates for:<br>a) Norway<br>b) Hordaland county where the the plant is located<br>c) internal plant group (all workers excluding ferrochromium workers) | 7<br>7<br>7 | 2.3<br>3.9<br>7.3* | all other sites | 16 | 0.8 | yes[a] |
| Axelsson et al., 1980 | Historical Prospective Cohort | Males employed for at least 1 year during 1930 - 1975 in a Swedish ferroalloy plant; FU of deaths reported since 1951 to the National Bureau of Statistics. | Cancer mortality rates for Älvsborg county in which the plant was located. | 5 | 0.7 | all other sites | 65 | 0.9 | yes[b] |
| Axelsson & Rylander, 1980 | Proportional Mortality Study | Population of Trollhättan and Vänersborg, communities in Älvsborg county which have ferrochromium-producing plants, for the period 1961 - 1975. | Population of Älvsborg county and communities therein, grouped according to size for the period 1961 - 1975. | No increase in mortality rate was found when comparisons were made with communities of the same size. | | | n/a | | yes[c] |

[a] Environmental chromium levels (in mg/m³) - Norwegian plant

| Operation | Total Cr | | |
|---|---|---|---|
| | Avg. | Range | N |
| Ferrochromium department (except packing) | 0.05 | 0.01-0.37 | 61 |
| Packing | 0.23 | 0.01-1.34 | 18 |

Air sampling was conducted in 1975. 11% to 33% of the chromium was water soluble.

[b] Environmental chromium levels (in mg/m³) - Swedish plant

| Operation | Est. levels of exposure | |
|---|---|---|
| | $Cr^{0}$+Cr(III) (insol.) | Cr(VI) (sol.) |
| Arc furnace | 2.5 | 0.25 |
| Transport, metal grinder, sampling | 0.5-2.5 | 0.01-0.05 |
| Maintenance | 2.5 | 0.05 |

Approximate calculations on exposure levels were made based on recent measurements and discussions with retired workers and foremen employed in the 1930's. General working conditions are known to have improved considerably over the 1970's. The authors state that the estimated exposure data should not be used to construct general dose-response relationships or to define threshold values. Exposure to Cr(VI) also occurred in chrome alum production from 1950-56.

[c] Environmental chromium levels (in mg/m³) - Swedish communities near plants.

| Community | Total Cr |
|---|---|
| Most polluted areas in Trollhättan & Vänersborg | $(0.1 - 0.4) \times 10^{-3}$ |
| Rural areas of Älvsborg | $(1-8) \times 10^{-6}$ |

Monthly readings were taken in 1976-79.

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* Statistical significance with $p < 0.05$

Table 4.6

Summary of Epidemiological Studies of Cancer in Workers Employed in the Chromium Plating Industries

| Reference | Study Design | Definition of Cohort, Follow-up (FU) and Analysis | Comparison Population | Respiratory Cancer |  | Other Cancers |  |  | Exposure Data |
|---|---|---|---|---|---|---|---|---|---|
|  |  |  |  | No. of Cases | Estimate of Relative Risk | Site | No. of Cases | Estimate of Relative Risk |  |
| Royle, 1975a | Historical Prospective Cohort | Current and former workers employed for more than 3 months in 54 British plants | Non-exposed workers individually matched on age, sex, and when last known to be alive. | 24 | 1.8 | G.I. | 11 | 2.8 | Yes$^a$ |
|  |  |  |  |  |  | Other sites | 16 | 2.3 |  |
| Waterhouse, 1975 | Historical Prospective Cohort | About 5000 workers, just over half being male, employed since 1946 in a British plant; FU incomplete | not specified | 49 (males) | 1.4* | Not reported |  |  | No |
| Okubu and Tsuchiya, 1977 | Historical Prospective Cohort | Workers with 6 months of employment in the Tokyo chromium plating industry and registered with a health insurance society between 1970 & 1976; FU through reports from plant managements (70% response) and FU of retirees | Tokyo mortality rates. Also compared with non-exposed chemical and unskilled workers. | 0 | - | Other sites | 5 | 0.5 | No |
| Franchini et al., 1983 | Historical Prospective Cohort | Males employed for more than 1 year prior to Jan. 1972 in 9 plants in Italy; FU for the years 1951-81: | Cancer mortality rates in Italian males |  |  |  |  |  |  |
|  |  | -overall |  | 3 | 3.8 | all sites | 7 | 2 | No$^b$ |
|  |  | -"hard" platers$^b$ |  | 3 | 5* |  | 6 | 2.7* |  |
| Waterhouse, 1980 | Historical Prospective Cohort | 2297 workers who commenced employment in 1946-70, worked for 6 months or more, and left the company by Dec. 1970; 13.6% not traced. FU for 1946-1980 for subcohort of non-bath workers: | Cancer mortality in England and Wales |  |  |  |  |  | No |
|  |  | -overall |  | 35 | 1.7* | stomach | 12 | 2.0* |  |
|  |  |  |  |  |  | larynx | 5 | 10.0* |  |
|  |  | -high exposure (3-8 y) |  | 12 | 3.6* | all sites | 20 | 2.6* |  |

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* Statistical significance with $p < 0.05$

a. Air sampling was conducted during 1969-70. Of the 54 plants in the study 42 were involved in the hygiene survey, with the others either having ceased operations or refusing permission.

| No. of Plants (vat type) | Cr Air Levels (mg/m$^3$) |
|---|---|
| 3 (automatic) | <0.005 in one; not detectable in the other 2 |
| 2 (manual) | >0.05 |
| 37 (mostly manual) | <0.02 |
| 12 (mostly manual) | not surveyed |

b. "Hard" platers were more exposed to chromic acid mist than were "bright" platers.

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# APPENDIX I

# SUMMARY OF BACTERIAL TESTS FOR MUTAGENICITY AND ALLIED CYTOGENETIC EFFECTS OF CHROMIUM COMPOUNDS

(Reproduced in whole from IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Some Metals and Metallic Compounds, Vol. 23, pp. 280-283 (1980). International Agency for Research on Cancer, Lyon, France.)

Table 9. Summary of bacterial tests for mutagenicity and allied effects of chromium compounds

| Bacterial assay system | Trivalent chromium[a] Test compound(s) | Result | Hexavalent chromium Test compound(s) | Result | Reference |
|---|---|---|---|---|---|
| Reverse mutation: | none tested | – | $Na_2CrO_4$; $CaCrO_4$; $K_2CrO_4$; | + | Venitt & Levy (1974) |
| *E. coli* WP2 | none tested | | $K_2Cr_2O_7$ | + | Nishioka (1975) |
| $trp^- \rightarrow trp^+$ | none tested | | $K_2CrO_4$ | + | Green *et al.* (1976) |
| Fluctuation test | none tested | | $PbCrO_4$; $CrO_3$ | + | Nestmann *et al.* (1979) |
| Plate test | none tested | | $PbCrO_4$; $CrO_3$ | – | |
| Reverse mutation: *E. coli* Hs30R $arg^- \rightarrow arg^+$ | $Cr(OOCCH_3)_3$ (high dose) | + | $K_2Cr_2O_7$; $K_2CrO_4$ | + | Nakamuro *et al.* (1978) |
| Reverse mutation: *S. typhimurium* (various strains) | $CrCl_3$ | – | $K_2Cr_2O_7$; $K_2CrO_4$ | + | Tamaro *et al.* (1975) |
| $his^- \rightarrow his^+$ | $CrK(SO_4)_2$; $CrCl_3$ | – | $Na_2Cr_2O_7$; $CrO_3$; $CaCrO_4$; $K_2CrO_4$ | + | Petrilli & De Flora (1977) |
| | $CrK(SO_4)_2$; $CrCl_3$ | –* | none tested | | Petrilli & De Flora (1978a) |
| | $Cr_2O_3.FeO$ (also contained Cr[VI]) (*addition of oxidizing agent made these trivalent compounds mutagenic) | + | | | |
| | none tested | | $Na_2Cr_2O_7$; $CrO_3$; $K_2CrO_4$; $Zn_2CrO_4(OH)_2$; $PbO.PbCrO_4$ (*mutagenicity suppressed by addition of rat liver microsomes, erythrocyte lysates and reducing agents) | +* | Petrilli & De Flora (1978b) |
| | none tested | | $Na_2Cr_2O_7$ (*mutagenicity decreased by preincubation with human gastric juice) | +* | De Flora *et al.* (1979) (abstract) |
| | none tested | | chromate and dichromate (*mutagenicity decreased by addition of rat liver microsomes) | +* | Löfroth (1978) |
| | chromic salts | – | chromate and dichromate | + | Löfroth & Ames (1978) |
| | none tested | | $PbCrO_4$; $CrO_3$ (*mutagenicity unaffected by addition of rat liver microsomes) | +* | Nestmann *et al.* (1979) |
| Forward mutation: *E. coli* K-12/343/113(λ) $gal^+$ | none tested | | $PbCrO_4$ | – | Nestmann *et al.* (1979) |
| Differential killing: ('DNA-repair') *B. subtilis* $rec^+/rec^-$ | $CrCl_3$ | – | $K_2Cr_2O_7$; $K_2CrO_4$ (*mutagenicity suppressed by addition of reducing agents) | +* | Nishioka (1975) |
| | $CrCl_3$ | – | | | Nakamuro *et al.* (1978) |
| | $Cr(OOCCH_3)_3$ | + | $K_2Cr_2O_7$; $K_2CrO_4$; $CrO_3$ | + | |
| | $Cr_2(SO_4)_3$ | – | $CrO_3$; $K_2CrO_4$; $K_2Cr_2O_7$ | + | Kada *et al.* (1980) |
| *E. coli* $polA^+/polA^-$ | none tested | | $PbCrO_4$ | – | Nestmann *et al.* (1979) |

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[a] Because most anhydrous Cr[III] compounds are insoluble, those listed in this table were used in various hydrated forms

Table 10. Summary of cytogenetic effects of chromium compounds on cultured mammalian cells

| Cell type | Trivalent chromium |  |  | Hexavalent chromium |  |  | Reference |
|---|---|---|---|---|---|---|---|
|  | Test compound | Dose range ($\times 10^{-6}$ M) | Result[a] | Test compound | Dose range ($\times 10^{-6}$ M) | Result[a] |  |
| Primary Syrian hamster embryo cells | $CrCl_3$<br>$Cr_2(SO_4)_3$ | 6.8-68<br>3.5-35 | –<br>– | $K_2Cr_2O_7$ (*effect abolished by addition of a reducing agent)<br>$CrO_3$ | 0.3-1.7<br>0.7-3.4 | +*<br>+ | Tsuda & Kato (1976, 1977) |
| Human peripheral blood lymphocytes | $CrCl_3$ | 1500-2255 | + | $CrO_3$ | 20-60 | + | Kaneko (1976) |
| Primary mouse fetal cells | $CrCl_3$ | 8 (as Cr) | + | $K_2Cr_2O_7$ | 0.3-2 (as Cr) | + | Raffetto *et al.* (1977) |
| Human embryonic fibroblasts | none tested |  |  | $K_2Cr_2O_7$ | 0.5 | + | Bigaliev *et al* (1977, 1978) |
| Human peripheral blood lymphocytes | $Cr(OOCCH_3)_3$<br>$CrCl_3$ | 16-32<br>32 | +<br>– | $K_2Cr_2O_7$<br>$K_2CrO_4$ | 0.5-4<br>4-8 | +<br>+ | Nakamuro *et al.* (1978) |
| Chinese hamster V79/4 cell line | none tested |  |  | $K_2Cr_2O_7$ | 1.7-2.7 | + | Newbold *et al.* (1979) |
| Fm3A (mouse mammary carcinoma) | $Cr_2(SO_4)_3$ | 32-1000 | – | $K_2CrO_4$<br>$K_2Cr_2O_7$<br>$CrO_3$ | 3.2-10<br>0.6-3.2<br>1-10 | +<br>+<br>+ | Umeda & Nishimura (1979) |
| Chinese hamster cell line CHO | $CrCl_3$<br>$CrK(SO_4)_2$<br>$Cr(OOCCH_3)_3$ | 960 (as Cr)<br>2885<br>96-385 | +<br>+<br>+ | $K_2Cr_2O_7$<br>$Na_2Cr_2O_7$<br>$K_2CrO_4$<br>$Na_2CrO_4$<br>$CrO_3$<br>$CaCrO_4$ | 2-20 (as Cr)<br>2-20 (as Cr)<br>5 (as Cr)<br>5-10 (as Cr)<br>2-5 (as Cr)<br>10 (as Cr) | +<br>+<br>+<br>+<br>+<br>+ | Levis & Majone (1979) |

[a] In all these studies, '+' indicates the induction of significant increases in chromatid breaks and fragments, and in some cases rearrangements.

# APPENDIX II

# SUMMARY OF CARCINOGENICITY STUDIES OF CHROMIUM AND CHROMIUM COMPOUNDS IN ANIMALS

(Reproduced in whole from IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Some Metals and Metallic Compounds, Vol. 23, pp. 264-273 (1980). International Agency for Research on Cancer, Lyon, France.)

Table 8. Summary of carcinogenicity studies of chromium and chromium compounds in animals

| Compound | Species | Route and dosage | Findings | Reference |
|---|---|---|---|---|
| Chromium powder | Mouse | 4 i.p. injections of 0.2 ml of a 0.005% solution | 1 myeloid leukaemia in 50 treated animals | Hueper, 1955 |
| | Mouse | 6 i.v. injections of 0.05 ml of a 0.005% solution | No tumours | Hueper, 1955 |
| | Mouse | 6 intrapleural injections of 0.2 ml of a 0.005% suspension | No tumours in 50 treated mice | Hueper, 1955 |
| | Rat | 1 intratracheal injection of 10 mg | No squamous-cell carcinomas of the lung in 12 treated rats | Mukubo, 1978 |
| | Rat | 1 i.m. injection of 2 mg | No local tumours in 22 surviving treated animals | Sunderman *et al.*, 1974 |
| | Rat | 6 i.p. injections of 0.1 ml of a 0.05% suspension | No increase in round-cell sarcoma incidence compared with controls; 2 insulinomas in treated animals, none in controls | Hueper, 1955 |
| | Rat | 6 i.v. injections of 0.18 ml of a 0.05% suspension | 2 rats with pulmonary adenomas; no increase in sarcomas compared with controls | Hueper, 1955 |
| | Rat | 6 intrapleural injections of 0.05 ml of a 33.6% (by weight) suspension or 6 intrapleural injections of 0.1 ml of a 0.5% suspension | 2 haemangiomas and 1 angiosarcoma in 50 treated animals and 0/25 controls | Hueper, 1955 |
| | Rat | Intramedullary injection into the femur of 46 mg | No injection-site tumours in 25 treated animals | Hueper, 1955 |
| | Rabbit | 18 i.v. injections of 0.5 ml/kg bw of a 5% suspension | 1 carcinoma of lymph node in 3 treated survivors and 0/4 controls | Hueper, 1955 |
| Unroasted chromite [III] ore | Mouse | Intrapleural injection of 10 mg in 0.5 ml distilled water | Granulomas | Davis, 1972 |
| | Rat | 6 intrapleural injections of 0.05 ml of a 73.4% (by weight) suspension | Injection-site sarcoma in 1/25 treated animals | Hueper, 1955 |
| | Rat | Intramedullary injection into the femur of 58 mg | No injection-site tumours in 25 treated rats | Hueper, 1955 |
| | Rabbit | 12 i.v. injections of 5 ml of a 5% suspension | No tumours | Hueper, 1955 |
| Roasted chromite [III] ore | Mouse | I.m. implantation of 10 mg (equivalent to 0.79 mg chromium) | No implantation-site tumours | Payne, 1960b |
| | Rat | I.m. implantation of 25 mg | Sarcomas at implantation site in 3/31 treated animals and 0 vehicle controls | Hueper, 1958 |
| | Rat | I.m. implantation | Tumours (type unspecified) at implantation site in 1/34 treated animals and 0/32 vehicle controls | Hueper, 1961 |
| | Rat | Intrapleural implantation | Tumours (type unspecified) at implantation site in 5/32 treated animals and 0/34 controls | Hueper, 1961 |

Table 8 (contd)

| Compound | Species | Route and dosage | Findings | Reference |
|---|---|---|---|---|
| Roasted chromite [III] ore (contd) | Rat | Intrapleural implantation of 25 mg | Lung squamous-cell carcinomas in 2/4 treated survivors and 1 lung adenoma in 1/4 controls | Hueper, 1958 |
|  | Rat | Intrapleural implantation of 25 mg (equivalent to 2 mg chromium) | Implantation-site tumours (type unspecified) in 3/35 treated rats and 0/35 controls | Payne, 1960b |
| Mixed chromate [VI] dust[a] | Mouse | Inhalation, 4 hrs/day, 5 days/wk for 16-58 wks (total dose chromium inhaled: 480-1205 mg-hrs) | No lung carcinomas; no significant increase in lung adenomas in 500 treated animals compared with controls | Baetjer *et al.*, 1959b |
|  | Mouse | 5-6 intratracheal instillations of dust (equivalent to 0.04 mg chromium trioxide) | No more lung tumours in 506 treated mice than in controls | Baetjer *et al.*, 1959b |
|  | Mouse | 4 intrapleural injections of 0.05 ml of a 2 or 4% suspension | No increase in lung tumour incidence in 55 treated animals compared with 41 controls | Baetjer *et al.*, 1959b |
|  | Rat | Inhalation, 4-5 hrs/day, 4 days/wk for lifespan (chromic oxide concentration of 3-4 mg/m$^3$) | No significant increase in tumour incidence in 78 treated rats compared with controls | Steffee & Baetjer, 1965 |
| Mixed chromate dust + potassium dichromate [VI] | Rat | 16 intratracheal injections of 0.1 ml of suspension of 0.5% roasted chromate + 0.6% potassium dichromate (equivalent to 0.07 mg chromium/dose) | No significant increase in tumour incidence compared with controls | Steffee & Baetjer, 1965 |
| Mixed chromate [VI] dust + potassium dichromate [VI] + sodium chromate [VI] + pulverized residue dust | Rabbit | Inhalation, 4 days/wk for 50 months | No increase in tumour incidence compared with controls | Steffee & Baetjer, 1965 |
|  | Guinea-pig | Inhalation, 4 days/wk for lifespan | Pulmonary carcinomas in 3/50 treated animals | Steffee & Baetjer, 1965 |
| Barium chromate [VI] | Rat | I.m. implantation of 25 mg | No implantation-site tumours in 35 rats | Hueper & Payne, 1959 |
|  | Rat | I.m. implantation | No implantation-site tumours in 34 rats | Hueper, 1961 |
|  | Rat | Intrapleural implantation | Implantation-site tumours in 1/31 treated rats and 0/34 controls | Hueper, 1961 |
| Calcium chromate [VI] | Mouse | Inhalation, 13 mg/m$^3$ 5 hrs/day, 5 days/wk for lifespan | Lung adenomas in 14/136 treated animals and 5/136 controls | Nettesheim *et al.*, 1971 |
|  | Mouse | I.m. implantation of 10 mg | Implantation-site sarcomas in 2/50 and in 0/50 controls | Payne, 1960a |
|  | Mouse | 1 s.c. injection of 10 mg | Injection-site sarcomas in 1/13 and in 0/52 controls | Payne, 1960a |

Table 8 (contd)

| Compound | Species | Route and dosage | Findings | Reference |
|---|---|---|---|---|
| Calcium chromate [VI] (contd) | Rat | Bronchial implantation | 6 squamous-cell carcinomas and 2 adenocarcinomas of the lung in 100 treated rats; 0/24 controls | Laskin *et al.*, 1970 |
| | Rat | Bronchial implantation | Increased incidence of bronchial squamous-cell carcinomas | Levy & Venitt, 1975 |
| | Rat | Inhalation, 2 mg/m$^3$, 589 exposures of 5 hrs over 891 days | 1 squamous-cell carcinoma of lung, 1 of larynx, 1 'peritruncal tumour' (no. of treated animals unspecified) | Laskin, 1972 |
| | Rat | I.m. implantation of 12.5 mg | Malignant tumours at implantation site in 4/8 treated animals | Hueper & Payne, 1962 |
| | Rat | 20 injections, total dose 19 mg | Injection-site sarcomas in 18/24 and 0 in vehicle controls | Roe & Carter, 1969 |
| | Rat | 12 injections of 4 mg | Injection-site sarcomas in 5/45 and 0/22 in vehicle controls | Furst *et al.*, 1976 |
| | Rat | I.m. implantation of 25 mg | Injection-site sarcomas in 8/35 treated animals and 0/32 controls | Hueper & Payne, 1959 |
| | Rat | I.m. implantation | Tumours (type unspecified) at implantation site in 9/32 treated animals and 0/32 controls | Hueper, 1961 |
| | Rat | Intrapleural implantation of 12.5 mg | Malignant tumours (unspecified) at implantation site in 8/14 treated animals | Hueper & Payne, 1962 |
| | Rat | Intrapleural implantation | Tumours (type unspecified) at implantation site in 20/32 treated animals and 0/34 controls | Hueper, 1961 |
| | Hamster | Inhalation, 2 mg/m$^3$ 589 exposures | 1 squamous-cell carcinoma and 1 papilloma of larynx (no. of treated animals unspecified) | Laskin *et al.*, 1972 |
| Sintered calcium chromate [VI] | Mouse | I.m. implantation of 10 mg | Implantation-site sarcomas in 9/46 treated animals and 0/50 controls | Payne, 1960a |
| | Mouse | S.c. injection of 10 mg | No injection-site sarcomas | Payne, 1960a |
| | Rat | I.m. implantation of 25 mg | Implantation-site sarcomas in 8/35 treated animals and 0 controls | Hueper & Payne, 1959 |
| | Rat | I.m. implantation | Tumours (type unspecified) at implantation site in 12/34 treated animals and 0/32 controls | Hueper, 1961 |
| | Rat | Intrapleural implantation | Tumours (type unspecified) at implantation site in 17/33 treated rats and 0/34 controls | Hueper, 1961 |
| Chromic [III] acetate | Mouse | P.o., 5 mg/l drinking-water for life | No increase in tumour incidence | Schroeder *et al.*, 1964 |
| | Rat | P.o., 5 mg/l drinking-water for life | No increase in tumour incidence | Schroeder *et al.*, 1965 |
| | Rat | 8 i.m. implantations of 25 mg each, over 24 months | Implantation-site sarcoma in 1/35 treated animals | Hueper & Payne, 1962 |
| | Rat | I.m. implantation | Implantation-site tumour (type unspecified) in 1/34 and in 0/32 controls | Hueper, 1961 |
| | Rat | 8 intrapleural implantations of 25 mg each over 13 months | No implantation-site tumours after 2 years in 42 treated animals | Hueper & Payne, 1962 |

Table 8 (contd)

| Compound | Species | Route and dosage | Findings | Reference |
|---|---|---|---|---|
| Chromic [III] acetate (contd) | Rat | Intrapleural implantation | Implantation-site tumour (type unspecified) in 1/34 and in 0/34 controls | Hueper, 1961 |
| Chromic [III] oxide | Rat | P.o., 1, 2 and 5% in bread on 5 days/wk for 2 years | 1% dose: 3/60 mammary fibroadenomas<br>2% dose: 1/60 " "<br>5% dose: 3/60 " "<br>controls: 1/60 mammary carcinoma; 2/60 fibroadenomas | Ivankovic & Preussmann, 1975 |
| | Rat | Single intratracheal application of 50 or 20 mg | 50 mg dose: 7/34 with tumours (4 with lung sarcomas)<br>20 mg dose: 6/18 with tumours (5 with lung sarcomas)<br>no controls | Dvizhkov & Fedorova, 1967 |
| | Rat | Bronchial implantation | No lung tumours in 98 animals | Laskin *et al.*, 1970 |
| | Rat | 1 i.p. injection of 20 mg | Lung sarcomas in 4/20; no controls | Dvizhkov & Fedorova, 1967 |
| | Rat | 2 intrapleural injections of 5 mg | Reticulum-cell sarcomas of lung in 3/17 treated animals; no controls | Dvizhkov & Fedorova, 1967 |
| Chromium carbonyl | Rat | Injection of 2.5 mg into subcutaneously implanted tracheal rings | Squamous-cell carcinomas in 2/22 animals; none in 4 vehicle controls | Lane & Mass, 1977 |
| Chromium [III] sulphate | Mouse | 24 i.p. injections (total doses: 480, 1200 and 2400 mg/kg bw) | No significant increase of pulmonary adenoma incidence in 60 treated rats compared with 40 vehicle and untreated controls | Stoner *et al.*, 1976 |
| Chromium [VI] trioxide | Rat | Bronchial implantation | No increase in lung tumour incidence in 100 treated rats compared with 24 controls | Laskin *et al.*, 1970 |
| Sintered chromium [VI] trioxide | Mouse | 1 s.c. injection of 10 mg | No injection-site tumours in 52 treated animals | Payne, 1960a |
| | Rat | I.m. implantation of 25 mg | Implantation-site sarcomas in 15/35 treated animals and 0/35 controls | Hueper & Payne, 1959 |
| Cobalt-chromium alloy | Rat | I.m. injection of 28 mg | Injection-site sarcomas in 7/74 treated rats; other tumours in 7/74 | Heath *et al.*, 1971 |
| Lead chromate [VI] | Mouse | 4 i.m. injections of 3 mg | 2 lymphomas and 3 lung adenocarcinomas in 17 mice necropsied; similar incidences in controls | Furst *et al.*, 1976 |
| | Rat | 1 s.c. injection of 30 mg | Injection-site sarcomas in 26/40 treated animals and 0/60 vehicle controls | Maltoni, 1974, 1976a |
| | Rat | 9 i.m. injections of 8 mg | Injection-site sarcomas in 31/47 treated rats; 3 renal carcinomas; 0/22 in vehicle controls | Furst *et al.*, 1976 |
| | Rat | I.m. implantation | Tumour (type unspecified) at implantation site in 1/33 treated rats and 0/32 controls | Hueper, 1961 |
| | Rat | Intrapleural implantation | Tumours (type unspecified) at injection site in 3/34 treated rats and 0/34 controls | Hueper, 1961 |
| Lead chromate [VI] oxide | Rat | 1 s.c. injection of 30 mg | Injection-site sarcomas in 27/40 treated rats and 0/60 vehicle controls | Maltoni, 1974, 1976a |

Table 8 (contd)

| Compound | Species | Route and dosage | Findings | Reference |
|---|---|---|---|---|
| Potassium chromate [VI] | Rat | Bronchial implantation | No increased incidence of lung tumours | Levy & Venitt, 1975 |
| Potassium dichromate [VI] | Rat | Bronchial implantation | No increased incidence of lung tumours | Levy & Venitt, 1975 |
| Sodium chromate [VI] | Rat | Bronchial implantation | No increased incidence of lung tumours | Levy & Venitt, 1975 |
| Sodium dichromate [VI] | Rat | 16 i.m. injections of 2 mg | No injection-site tumours | Hueper & Payne, 1962 |
|  | Rat | I.m. implantation | No implantation-site tumours | Hueper, 1961 |
|  | Rat | 16 intrapleural injections of 2 mg | 1 lung adenocarcinoma in 39 treated animals; no injection-site tumours in 60 vehicle controls | Hueper & Payne, 1962 |
|  | Rat | Intrapleural implantation | No injection-site tumours in 26 treated animals | Hueper, 1961 |
|  | Rat | Bronchial implantation | No increase in lung tumours | Levy & Venitt, 1975 |
| Strontium chromate [VI] | Rat | I.m. implantation | Implantation-site tumours in 15/33 treated animals and 0/32 controls | Hueper, 1961 |
| Zinc potassium chromate [VI] | Rat | Bronchial implantation | Increased incidence of bronchial squamous-cell carcinomas | Levy & Venitt, 1975 |
| Zinc yellow | Mouse | 6 intratracheal injections of 0.03 ml of a a 0.2% suspension | No pulmonary carcinomas; pulmonary adenomas in 31/62 treated animals and 7/18 untreated controls | Steffee & Baetjer, 1965 |
|  | Rat | I.m. implantation | Tumours (type unspecified) at implantation site in 16/34 treated animals and 0/3 2 controls | Hueper, 1961[b] |
|  | Rat | Intrapleural implantation | Tumours (type unspecified) at implantation site in 22/33 treated animals and 0/34 controls | Hueper, 1961[b] |

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[a] See footnote on p. 254.

[b] It was not specified whether this compound was zinc chromate, zinc potassium chromate or zinc yellow.

# APPENDIX III

# PRINCIPLES AND PROCEDURES FOR THE INTERPRETATION OF EPIDEMIOLOGICAL STUDIES

(Reproduced in whole from Fifth Annual Report, April 1, 1982 to March 31, 1983, pp. 136-138. Advisory Council on Occupational Health and Occupational Safety, Ontario Ministry of Labour, Toronto, Ontario).

*Occupational Mortality Studies*

There are six different study designs that could be employed to conduct research on the incidence of mortality in the workplace. These designs, which are set out in Figure 1, typically produce results of a fixed inherent quality. The appendix, which is attached to this document, provides a full description of each study design, assesses the strengths and weaknesses of each approach, and provides criteria from which to evaluate the quality of individual research papers that fall within a specific design category. It should be noted, at this stage, that approximately 95 per cent of all occupational mortality studies follow either the historical prospective cohort or proportional mortality design. As a result, the appendix will place extra emphasis on a discussion of these two study types.

In Figure 1, the advisory council has also set out a scale for ranking the quality of evidence obtained from different classes of occupational mortality study designs. As can be seen from the diagram, the evidence obtained from a randomized control trial is considered to possess the highest level of inherent quality. As one moves to less stringent study designs, however, the quality of the results obtained tends to diminish.

According to accepted epidemiological practice, a research paper that falls into a design category on the high end of the scale will almost always be preferred to a study whose design category appears closer to the bottom of the ranking. There may exist situations, however, where a study with a strong design may be so flawed in some respects that it ought not to be used as a basis for setting occupational health standards. If this is the case, then another study with an inferior design might be the only source available from which one could draw an association between exposure to a particular agent and a health risk.

Although study designs at the low end of the scale suffer from major deficiencies in design, the observations culled from these research materials often provide useful first stage assessments of the adverse health effects that could be produced by an environmental agent. As an example, case series studies provided the first suggestive evidence that angiosarcoma of the liver was caused by occupational exposure to vinyl chloride. When the results of a study with a low ranking design indicate that a particular agent may pose a threat to worker health, the next step could involve i) initiating immediate regulatory action and/or ii) undertaking more sophisticated epidemiological studies.

In addition to specifying guidelines for the ranking of different categories of occupational mortality studies according to their design, there is also a need to set out criteria that regulators might employ to evaluate the quality of individual studies that fall within the same generic category. Frameworks for assessing the strengths and weaknesses of various study types have been described in the literature.[1] The advisory council has drawn on these materials in an attempt to develop a comprehensive set of criteria for evaluating the two most common classes of study designs encountered in the occupational mortality field (i.e. historical prospective cohort and proportional mortality studies). These criteria are set out in Tables 1 and 2, and are analysed in detail in the accompanying appendix. A framework for the evaluation of the case control study (a design that is useful for the study of rare diseases) will not be set out since this approach tends to be employed infrequently in the context of occupational epidemiology.[2]

**Table 1: Criteria For Evaluating The Quality of Historical Prospective Cohort Studies**

1) **Definition of the Study Cohort**
    - a) Method of Selection
    - b) Criteria for Inclusion or Exclusion
    - c) Extent to Which All Cohort Members Were Considered
    - d) Size of the Cohort

2) **Quantification of Exposure**
    - a) Detail in Work History Files
    - b) Extent of Industrial Hygiene Data
    - c) Quality of Industrial Hygiene Data
    - d) Consideration of Confounding Factors

3) **Ascertainment of Mortality**
    - a) Quality of Follow-up Technique
    - b) Completeness of Follow-up
    - c) Availability of Death Certificates or Other Records to Determine the Cause of Death
    - d) Length of Follow-up

4) **Analysis of Results**
    - a) Suitability of the External Control Group
    - b) Availability of an Internal Control Group
    - c) Allowance for Latency Effects
    - d) Use of Appropriate Statistics
    - e) Demonstration of an Exposure Gradient
    - f) Discussion of Possible Biases

**Table 2: Criteria For Evaluating The Quality of Proportional Mortality Studies**

1) Extent to Which All Deaths in the Study Group Have Been Recorded.

2) Method by Which Deaths Are Classified.

3) The Basis for Choosing a Control Group.

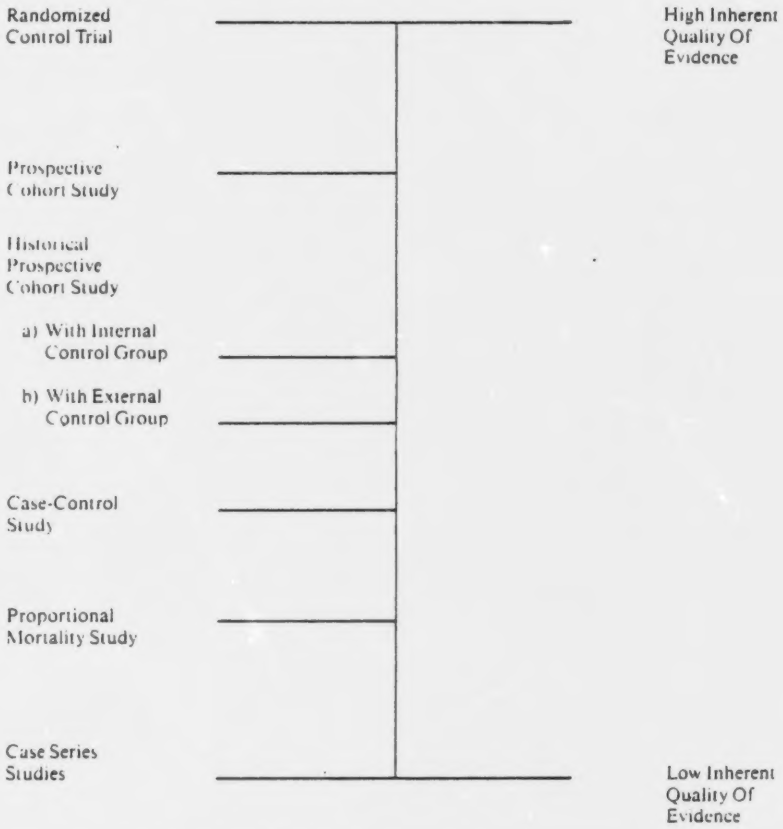
4) Classification of Mortality Data by Age and Calendar Time of Death.

5) Elimination of Obvious Competing Risks.

6) Illustration of an Exposure Gradient.

7) Allowance for Latency Effects.

**Figure 1:** **The Quality of Evidence Typically Associated with Different Classes of Occupational Mortality Study Designs**


Randomized Control Trial
High Inherent Quality Of Evidence
Prospective Cohort Study
Historical Prospective Cohort Study
a) With Internal Control Group
b) With External Control Group
Case-Control Study
Proportional Mortality Study
Case Series Studies
Low Inherent Quality Of Evidence

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# 5. CHROMIUM HYPERSENSITIVITY

## 5.1 Introduction

In this chapter, hypersensitivity effects associated with chromium exposure are reviewed.

The concept of hypersensitivity, as it is referred to here, conforms to that of Pepys, the eminent immunologist, in his definition of "allergy": "the profound biologic transformation of the intact organism resulting from adequate exposure to viable and non-viable agents, so that re-exposure, often too small, indeed often minute, and apparently innocuous amounts of extrinsic agents, elicits a different sort of reaction (than that occurring at first exposure)..." (Pepys, 1977). Pepys further describes the traditional criteria for regarding a reaction as hypersensitivity-mediated (with or without supporting immunologic evidence):

1. history of previous exposure (i.e., a period of sensitization);
2. a tendency for the specific sensitivity to increase with further exposure;
3. a low (usually) proportion of affected individuals in the exposed population.

The hypersensitivity phenomena associated with chromium exposure that are considered in this chapter are generally consistent with this definition and these criteria.

Most of the literature on hypersensitivity induced by chromium compounds concerns skin allergies. Thus, the following discussion of prevalence, clinical patterns, specific known causes, prognosis, prophylaxis, and pathogenesis, has a dermatologic focus. The available evidence concerning respiratory hypersensitivity effects of chromium is also summarized.

## 5.2 Cutaneous Chromium Hypersensitivity - Clinical Considerations

### 5.2.1 Prevalence

Chromium is generally accepted as being the second most common skin allergen in the general population, after nickel (Polak, 1983); in men, it is the most frequent sensitizer ( Cronin, 1980 ). Although data on the prevalence of chromium sensitivity in the general population are scarce, numerous reports cite the proportion of patients investigated for contact dermatitis who reacted positively to patch testing with 0.5% potassium dichromate. In these studies, the prevalence of patch test sensitivity to chromate showed wide variability, perhaps because the breakdown of subjects by sex was not reported. Table 5.1 shows the prevalence of chromate sensitivity found in six important investigations in which sex was taken into account. The greater frequency of chromate sensitivity among males is apparent. Both differing sex ratios of prevalence and differing overall rates by geographical setting have been attributed largely to the proportion of the local population engaged in the building industry, particularly under hot humid conditions. In North America

(North American Contact Dermatitis Group, 1973), considerable variability in prevalence was observed among study sites (Table 5.2). No comprehensive study of the extent to which chromate sensitivity occurs among Canadian contact dermatitis patients has been conducted.

The age distribution of chromate-sensitive patients seen at St. John's Hospital for Diseases of the Skin in London, England, appears to correspond to the working years of life (Table 5.3). Within given geographical settings, the prevalence of positive patch tests to chromate has remained reasonably constant over the years (Baer *et al*., 1964; Hammershoy, 1980). Moreover, there is no evidence of changing frequency patterns within sex categories (Cronin, 1980).

At St. John's Hospital, the existence of concomitant sensitivity to cobalt and/or nickel was also documented among patients with chromate sensitivity. As can be seen from the data in Table 5.4, the large majority of subjects reacted to chromate only.

### 5.2.2 Clinical Patterns

The hands are involved in the majority of chromate dermatitis cases. Typically affected, as with other contact sensitizers, are fronts of wrists, backs of hands, sides and backs of fingers, and finger webs (Burrows, 1983). Not infrequently, however, other parts of the body are involved and there may be a striking resemblance to other dermatitides. For example, among 134 patients with cement dermatitis, Burrows and Calnan (1965) observed patterns resembling nummular dermatitis in 13%, seborrheic dermatitis in 10% and stasis dermatitis in 7%. The palms were involved in 9% of cases. Fourteen individuals with positive patch tests to potassium dichromate were reported by Shanon (1965) to have a rash closely resembling atopic dermatitis; these patients improved after removal from chromate exposure. Engel and Calnan (1963) noted a number of cases of chromate dermatitis associated with primer paint exposure which were indistinguishable from seborrheic, nummular, or atopic dermatitis, in distribution and clinical appearance.

## 5.3 Associated Occupational and Environmental Exposures

### 5.3.1 Introduction

In 1925, chromium dermatitis was first reported (Parkhurst, 1925) in a blue print production worker with dermatitis who reacted positively to patch testing with 0.5% potassium dichromate. Since then, reports linking dermatitis with chromium exposure in diverse occupational and environmental contexts have multiplied considerably. However, the occupational or other possible sources of chromate exposure have been infrequently investigated among chromate-sensitive patients. Among 140 such subjects, Pirilä (1954) noted a predominance of cement workers (57%), with 11% being lithographers and photographers and 7% plating and metal workers. Among another series of 245 cases (Kresbach, 1967), exposure to cement in 27% was the only clear-cut category. At St. John's Hospital for Skin Diseases (Cronin, 1980), workers from building trades (46%) again figured prominently among patients with chromate sensitivity. Among female patients, about one-third had domestic work as a plausible source of exposure. Significantly, no source was found in a large proportion of both male (16%) and female subjects (44%).

Most of the information that we have concerning the allergenic risks of chromium derives from case and case series reports. Table 5.5 lists the major sources of occupational and environmental chromium exposure that have been associated with the development of contact sensitivity; the chromium compounds considered responsible are also shown.

The important features of these occupational and environmental exposures and their observed effects are briefly examined in this section. Extensive reviews of these topics have recently appeared (Cronin, 1980; Burrows, 1983).

### 5.3.2 Cement

Cement is the most important cause of chromate allergy and its role in producing dermatitis has long been recognized. In 1700, Ramazzini noted that "lime makes the hands of brick layers wrinkle and sometimes ulcerates them" (cited in Burrows, 1983). More than thirty years ago, Jaeger and Pelloni (1950) showed that 30 of 32 cement dermatitis patients reacted positively on patch testing to potassium dichromate, whereas only 5% of other patients did so. These investigators confirmed the link between cement dermatitis and chromium allergy, by demonstrating the presence of chromium in cement. Burrows (1978) has reported a declining rate of cement exposure among patients with chromate sensitivity, and he postulated that this may reflect changing methods of material handling in the building industry.

Cement is a skin irritant because of its alkaline, abrasive and hygroscopic properties. In the dry form, it is rarely associated with the development of dermatitis (Calnan, 1960). When cement becomes wet, by the addition of water or by absorbing it from skin, alkaline calcium hydroxide is liberated. The abrasiveness of cement increases as it absorbs water and when it is mixed with sand.

The trivalent chromium compounds present in cement precursors are oxidized to the hexavalent form during heating in kilns at temperatures exceeding 1000°C. The chromate content of cement has been measured by numerous investigators, and significant amounts have been found to be present with considerable consistency (Cronin, 1980). For example, Fregert and Gruvberger (1972) detected measurable quantities of water-soluble chromate (1-40 $\mu g\,Cr\,g^{-1}$ in all of 52 cement samples tested, although only in 2 were concentrations in excess of 35 $\mu g\,Cr\,g^{-1}$. Perone _et al_. (1974) found 18 of 42 samples of Portland cement to contain measurable water-soluble chromate, in amounts ranging from 0.1 to 5.4 μg Cr $g^{-1}$. Possible chromate sources in finished cement include:

1) the ingredients -- chalk, clay, and gypsum;
2) chromium-containing refractory bricks;
3) chromium-containing steel grinders;
4) ash.

It should be noted, however, that cement may contain numerous additives, of which some may be sensitizers or irritants in their own right (Calnan _et al_., 1969).

Cement dermatitis is a major occupational disease. In Europe in recent years, it has consistently constituted more than 25% of occupational dermatoses (Cronin, 1980). As already indicated (Section 5.3.1), nearly half of chromate-sensitive men examined by Cronin (1980) were likely to have acquired their sensitivity from cement. Cross-sectional studies in Scandinavia found approximately 5% of construction workers to have cement dermatitis (Wahlberg, 1969; Høvding, 1970, cited in Cronin, 1980). Burrows (1972) has estimated that 3.5% of workers in the building industry develop a dermatitis which leads to time off work. In European studies, over 75% of men with cement dermatitis have been found to react positively upon patch testing to chromate (Høvding, 1970, cited in Cronin, 1980).

Data concerning cement dermatitis in North America are limited. Although Perone _et al_. (1974) found that among 95 construction workers more than one-third had manifestations of mild to moderate dermatitis, only one reacted positively to patch testing with 0.25% dichromate. In contrast, between 2.5 and 6% of European cement workers with normal skin gave positive patch tests (Cronin, 1980).

### 5.3.3 Anti-Corrosion Agents

Chromium-associated dermatitis has been found in relation to exposure to anti-rust and anti-corrosion agents in a number of settings. A water-soluble chromate (usually potassium dichromate) is the active ingredient. For example, chromate-containing primer paint has been linked with the development of dermatitis in aircraft workers (Hall, 1944), and in workers who were wet-sandpapering car bodies (Engel and Calnan, 1963). Adams *et al*. (1976) reported that the content of water-soluble chromate in primer paints formulated with zinc potassium chromate was of the order of that found in patch test solutions, and much greater than that in cement. Chromate is currently being excluded from paints because of the carcinogenic risk during manufacturing (Burrows, 1983; also see Chapter 4).

The use of sodium dichromate as an anti-corrosive in coolants has been associated with the development of chromate dermatitis, particularly among diesel maintenance workers (Winston and Walsh, 1951; Calnan and Harman, 1961; Guy, 1954). The replacement of chromate by other anti-corrosives in coolant systems has been associated with a decline in chromate dermatitis in these circumstances.

The handling of chromate-treated galvanized sheets has also been linked to the development of contact dermatitis by Fregert _et al_. (1970a). These investigators demonstrated a recovery of between 1 and 5 mg chromium per person (about 3% was hexavalent) from the hand-wash water of workers handling galvanized sheets. This amount was much greater than that found in hand washes from bricklayers using cement (0 - 7 µg). Earlier, Newhouse (1963) reported 47 cases of chromate sensitivity among 130 car assembly workers, who were handling chromate-treated zinc-plated parts.

### 5.3.4 Bleaches and Detergents

In some countries, chromate in detergent and bleaches contributes substantially to the prevalence of hand dermatitis among those engaged in domestic work. A well recognized source of chromium allergy has been bleach (l'eau de Javel) to which chromate has been added for stabilizing and colouring purposes (Rabeau and Ukrainczyk, 1939; Hilt, 1954). Chromate has been removed from l'eau de Javel in France, but, as of 1973, not in Italy, Spain or Belgium (García-Pérez _et al_., 1973). Feuerman (1969) has attributed a high prevalence of chromate sensitivity among housewives with hand eczema, to the use of both bleaches and detergents. Subsequently, García-Pérez _et al_. (1973) have linked a declining frequency of chromate-positive hand dermatitis in female domestic workers to reduced quantities of chromate in detergents and bleaches. Over the same time period, no change in the rate of chromium sensitivity in men was observed. Apparently, chromate is not added to bleach in North America (Burrows, 1983).

### 5.3.5 Leather

Chromium compounds used in leather tanning have been extensively associated with sensitization of both tannery workers (Morris, 1958; Fregert, 1975; Bang Pedersen, 1982; Burrows, 1983) and the users of leather materials (Pirilä and Kilpiö, 1954; Rudzki and Kozlowska, 1980; Angelini *et al*., 1980). Morris (1958) reported four individuals with shoe dermatitis as reacting positively to patch testing with 0.2% basic (trivalent) chromic sulfate, used in the one-bath tanning process (involves Cr(III) only). Samitz and Gross (1961) have reported the presence of both trivalent and hexavalent chromium in extracts of leather with human sweat. However, Fregert and Gruvberger (1970) were able to identify only trivalent chromium in synthetic sweat and water extracts from shoe leather. Most of the chromium compounds used in the tanning process are Cr(III) derivatives (Stern, 1982). However, in the two-bath process used to prepare specialty leather, the skins are first treated with dichromate, which is followed by a reduction step which converts absorbed Cr(VI) to Cr(III). It should be noted that foot dermatitis can be associated with contact allergy to a number of other, non-chromium, shoe components (Cronin, 1966). The replacement of chrome-tanning by vegetable-tanning of gloves has markedly reduced their chromium content (Fregert and Gruvberger, 1979), but the impact of this change on the prevalence of hand dermatitis has not been reported.

### 5.3.6 Machine Oils

Exposure to chromate in unused (Holz *et al*., 1961; Calnan, 1978) and used (Weiler, 1969; Fregert and Gruvberger, 1976a) mineral oil has been associated with contact hypersensitivity manifestations. Rycroft (cited in Burrows, 1983) documented an increase in the level of chromium in soluble oil used in lathe machines over a period of five weeks; however, none of 70 operators reacted positively on patch testing to chromate.

### 5.3.7 Dyes

Chromate used in dyeing applications has been associated with the development of hypersensitivity in an upholstery factory worker (Fregert and Gruvberger, 1976b), card players in contact with green felt tables (Fregert *et al*., 1970b; Fisher, 1976), and the wearers of green military textiles (Fregert *et al*., 1978). Skin inflammation has also been reported in association with the green, chromium(III) oxide pigment in tatoos (Scutt, 1966; Cairns and Calnan, 1962).

### 5.3.8. Welding

Exposure to welding fumes has been reported in association with contact dermatitis of the face (Fregert and Övrum, 1963). Inhalation of welding fumes was linked to repeated episodes of dermatitis in the hands of a chromate-sensitive worker (Shelley, 1964). Chromium is commonly present in the coating and core of welding rods as well as in the workpiece. The latter is a minor source of fumes. Particularly, manual metal arc welding of stainless steel liberates considerable amounts of chromium, 90% of which is hexavalent and water soluble (Stern, 1982; see Section 4.4.2.3). Gas-shielded (inert) welding of stainless steel also liberates significant quantities of chromium, but a smaller fraction (~5%) is chromium(VI).

### 5.3.9 Foundry Sand, Boiler Linings, Ashes

As in chromate dermatitis associated with cement, much of the allergenicity of chromium exposure in certain other occupational settings is attributable to conversion of trivalent chromium into hexavalent forms, during heating under alkaline conditions. Thus, hand dermatitis associated with chromate sensitivity has developed in men working with moulding sand in foundries (Hellier, 1962). Investigation has revealed the source as the presence in foundry sand of ground chromium magnesite bricks, previously used as a refractory material (Fregert, 1963). Chromate dermatitis has also been reported in a fitter who was repairing the refractory lining of a boiler (Rycroft and Calnan, 1977). And finally, Fregert (1962) observed positive patch tests in chromium sensitive individuals elicited by water extracts of wood ashes. Unlike the ashes of coal, coke and fuel oil, wood ashes are alkaline and contain hexavalent chromium.

### 5.3.10 Printing

Following the report of Parkhurst (1925), a risk of chromate dermatitis had been recognized in the printing industry. Pirilä and Kilpiö (1954) reported chromate sensitivity in 27% of 149 cases of dermatitis among printing workers. Of 76 cases of dermatitis reported among lithographers (Levin et al., 1959), 17 patch tested positively to potassium dichromate. Spruit and Malten (1975) demonstrated high chromium levels in the materials handled by three men working in an offset printing factory, and who developed dermatitis and chromate sensitivity. The use of potassium dichromate in the printing industry has declined following suitable substitution by other agents (Burrows, 1983).

### 5.3.11 Chromate Production and Chromium Plating

The chromate production and chromium plating industries are a high-risk environment for chrome ulcerations (Gafafer, 1953; Anon, 1963; Henning, 1972). However, sound prevalence data are not available with regard to chromate sensitization in this setting. Royle (1975) found 25% of 997 electroplaters to have dermatitis. However, patch testing was not performed, and the reported prevalence was based on answers to a questionnaire. Among 897 chromate production workers examined, Gafafer (1953) noted 17 individuals with chrome dermatitis of the erythematous and papular type. They attributed this low prevalence to the existence of adequate washing facilities and the availability of clean working clothes. The possibility of the development of tolerance was not discussed. It is of interest to note that Edmundson (1951) found that only 2 of 56 chromate workers with chrome ulcers had positive patch tests to 0.5% of potassium dichromate. In other studies, little association has been observed between the finding of chrome ulcerations and the presence of chromium contact sensitivity (Cronin, 1980; Burrows, 1983).

### 5.3.12 Matches

Chromium in matches has been associated with dermatitis both in match factory employees (Pirilä, 1954) and in match users (Fregert, 1961; Rudzki and Kozlawska, 1980). Potassium chromate and dichromate are added to match heads as oxidizing agents.

### 5.3.13 Miscellaneous Industrial Exposures

Finally, mention is made of a number of miscellaneous sources of chromium exposure that have been associated with chromate dermatitis. Cumulatively, their impact is likely to be large.

Contact dermatitis attributed to chromium compounds has been reported in the pulp and paper industry (Fregert et al., 1972; Connor, 1972). Burrows (1983) has observed chromate dermatitis in association with exposure to timber preservatives. Chromate sensitivity (on patch testing) and dermatitis have also been demonstrated in laboratory workers involved in analyzing the fat and protein content of milk samples preserved with potassium dichromate (Huriez *et al*., 1975; Rogers and Burrows, 1975; Rudzki and Czerwinska-Dihnz, 1977). In an equally unusual situation, chromate sensitivity and dermatitis developed in a laboratory technician utilizing 10% potassium chromate as an indicator in determining the sodium chloride content in food (Bang Pedersen, 1977). Contact dermatitis has also been associated with exposure to chromium-containing glue (Morris, 1955). Among 26 workers involved in making television screens in a process using ammonium dichromate, 18 were found to have chromate dermatitis (Stevenson, 1975). Krook *et al*. (1977) reported dermatitis in a telegraphist who regularly handled magnetic tapes containing chromium(IV) oxide; patch testing revealed sensitivity to cobalt and nickel, as well to chromate. A final instance deserving mention concerns a tire fitter who developed hand dermatitis from chromate dissolved in a solution used to facilitate fitting the tire to the rim (Burrows, 1981).

### 5.3.14 Comment on Dose-Response

In most of the reports cited above that included sufficient subjects to allow an assessment of dose-response relationships for induction and elicitation of sensitivity, the concentrations and types of chromium compounds present were documented inadequately for this purpose. Generally speaking, there is little evidence that the prevalence of contact dermatitides correlates with dose. On the other hand, separate, detailed investigations of chromium speciation have been undertaken for a number of problematic exposure circumstances, such as cement use (Johnston and Calnan, 1958). It is obvious from such studies, that the hexavalent chromate ion is the most potent sensitizer. From the evidence cited, it is apparent that in numerous cases chromate dermatitis developed on contact of the skin to relatively low concentrations of chromate. It is concluded that the presence of chromium-containing compounds in the workplace, particularly in their hexavalent forms, should be cause for concern with respect to their sensitizing potential. As reviewed in Section 5.6, there is also considerable evidence that Cr(III) compounds are allergenic, although of inferior eliciting capacity in epicutaneous applications when compared to hexavalent chromium.

## 5.4 Prognosis

The consensus is that the outlook for persons with chromium dermatitis is bleak. In follow-up postal surveys, the majority of chromium dermatitis patients report on-going skin symptoms (Harrison, 1979; Dooms-Goossens et al., 1980). After four to seven years' follow-up in 48 patients, positive patch testing persisted in 38 individuals; in 27 subjects, chronic dermatitis was present; in 16, it was recurring; and in only five cases had the dermatitis cleared. In a 10 year follow-up of occupational chromium dermatitis, Fregert (1975) found only 7% of women and 10% of men to have healed (compared to 16%

and 24%, respectively, for dermatitis of all causes). Burrows (1972) reported that only 8% of individuals with cement dermatitis had cleared after 10 to 14 years from initial diagnosis, compared to 25% of those with other industrial dermatoses. Healing of cement dermatitis has been reported -- while the workers continued in their trade -- in 4 out of 46 building workers (Geiser and Girard, 1965) and 8 out of 17 brick layers (Hunziker and Musso, 1960).

The chronicity of chromium dermatitis is attributable to a number of factors: the persistence of hypersensitivity; the ubiquity of chromium compounds in foods and in objects coming into cutaneous contact; the persistence of chromium compounds in skin; and the co-existence of other contact allergies -- for example, to cobalt and nickel (Fregert and Rorsman, 1966) or rubber in rubber gloves (Fregert, 1975).

## 5.5 Prophylaxis

In this section, strategies for primary prevention of chromium dermatitis are outlined. Firstly, maintenance of workplace chromium exposure to the lowest feasible levels, good housekeeping, and a high level of awareness among workers and management are important. And secondly, numerous alternatives to the use of hexavalent chromium in industry have been sought and are available. For example, nitrites and amines can replace chromates as anti-rust agents. It is also possible to employ trivalent chromium in plating processes. Furthermore, chromium(III) salts may be replaced in part by aluminum(III) salts in tanning (Covington and Sykes, 1981), and a non-chromium, vegetable-tanning process is also possible. Combinations of aluminum salts and vegetable tannins are currently employed in some countries (Sykes et al., 1980). The use of ferrous sulphate in cement (to reduce hexavalent to trivalent chromium) also holds considerable promise. In a number of other industries, similar replacements or additions have been implemented.

## 5.6 Pathogenesis

### 5.6.1 Introduction

While the causal role of certain chromium compounds in inducing and eliciting contact dermatitis is undisputed, the allergenicity of chromium containing substances varies considerably. With regard to research into the elicitation of sensitivity reactions, Polak (1983) states this in the converse fashion: "Under favourable conditions such as a high degree of hypersensitivity, appropriate solvents, sufficiently long exposure time, etc., a positive skin reaction to all chromium compounds used (is) observed even if different concentrations of them are necessary".

The main determinants of the sensitizing and eliciting capacity of chromium compounds are generally considered to be valency, solubility, concentration, pH and presence of organic matter (Fregert, 1981). Our discussion of the pathogenesis of chromium hypersensitivity begins with a consideration of how the influence of these variables is mediated, with emphasis on the part played by valency. Then, the role of certain significant modulating factors is reviewed, namely: genetic influences, non-cutaneous absorption, and interaction of light. Lastly, relevant aspects are reviewed with respect to the phenomena of tolerance, desensitization and flare reactions. As a conceptual aid, provided first is a brief synopsis of our earlier discussions concerning the interaction of chromium compounds with tissues (Section 3.3.6).

### 5.6.2 Relevant Chemical Principles

The chromate ion is stabilized in alkaline solutions and therefore is less oxidizing. It readily penetrates skin and cell membranes and eventually is reduced to the Cr(III) ion by skin and intracellular components (see Sections 3.2.2.4, 3.3.4.3 and 3.3.5). In contrast, the hydrated Cr(III) ion, $Cr(H_2O)_6^{3+}$, does not penetrate skin well and appears largely to be excluded from cells. The lack of skin permeability is explained by the strong binding of this cation to skin constituents, especially proteins, and by its propensity to polymerize extensively due to hydrolysis (see Section 3.2.3.2). The latter process becomes serious at neutral pH values, and increases with increasing pH. Both types of interactions may be expected to reduce the ability of Cr(III) species to diffuse through the epidermis and dermis (see Section

3.3.6). It is also clear that the nature of the Cr(III) salt can have considerable bearing on the extent of binding and hydrolysis, and thus penetration. For example, the commercially purchased chromium(III) chloride on dissolution yields a singly-charged chloride complex, $[CrCl_2(H_2O)_4]^+$, rather than the triply-charged hydrated ion obtained by dissolving Cr(III) nitrate or sulphate (see Section 3.3.6). Basic chromic sulphate produces a doubly-charged species, $[Cr(H_2O)_5OH]^{2+}$. Such complex cations bear a lower net charge, and may be expected to be less reactive and to diffuse considerably better through skin. Furthermore, the state of hydration of chromium(III) salts often determines water solubility (e.g. anhydrous $CrCl_3$ is insoluble, while the hydrate $[CrCl_2(H_2O)_4]Cl \cdot 2H_2O$ is; see Section 3.2.3.3).

### 5.6.3. The Role of Valency: Clinical and Experimental Observations

#### 5.6.3.1 Human Studies

Controversy surrounds the issue of the valence state in which chromium participates in the formation of the antigenic determinant. Chromium has at least six valence states (see Section 3.2.1). However, only tri- and hexavalent compounds are stable enough to be considered as potential haptens (Burrows, 1978). The metal itself is considered non-sensitizing, and this lack of reactivity is attributed to a surface layer of insoluble chromium oxide (Cronin, 1980).

Early clinical experience suggested that only hexavalent compounds were associated with the induction of hypersensitivity (Jaeger and Pelloni, 1950; Calnan, 1960). However, the occurrence of contact allergy to trivalent chromium was demonstrated subsequently as well (Fregert and Rorsman, 1964; Rudzki *et al*., 1978). Nevertheless, while both valence states have been associated with the induction and elicitation of hypersensitivity reactions, it is generally recognized that the hexavalent form is the more potent agent in these functions; and clinical experience remains consonant with this perspective. In an experiment with volunteers, Kligman (1966) found that, upon epidermal administration by the 'maximization procedure', about half of a group of 23 human subjects became sensitized to trivalent chromium salts (chromium(III) oxide and chromium sulphate), whereas potassium dichromate sensitized each of 23 individuals. With respect to elicitation of sensitivity, Zelger (1964) demonstrated that the threshold concentrations for the production of positive skin tests in hypersensitive patients were generally much lower for hexavalent than trivalent compounds (see Table 5.6). Thus in five subjects reacting positively to 0.25% potassium dichromate (Samitz and Shrager, 1966), only one showed a (slightly) positive patch test reaction to 5% chromium(III) trichloride and 5% chromium(III) sulphate. None reacted to chromium(III) nitrate. Anderson (1960a) found that, of 19 patients reacting positively to patch testing with potassium dichromate, only one was positive to testing with the trivalent chloride at comparable chromium concentrations. On the other hand, other investigators observed that 11 of 17 chromium sensitive subjects reacted positively to epicutaneous tests with 0.5 M chromium trichloride (Fregert and Rorsman, 1964), although only four of 22 responded to this compound at a concentration of 0.07 M. Subsequently, it was found that 70 of 90 dichromate-positive subjects gave positive patch test reactions to 0.1 M chromium(III) lactate (Fregert and Rorsman, 1965), in which the active ingredient appears to be $CrL_2^+$ (with L denoting lactate; Morris, 1958). Forstrom et al. (1969) noted that 23 of 46 dichromate positive patients reacted positively to trivalent chromium in 1 to 5% solutions. It has been demonstrated (Rudzki et al., 1978) that differences in positive reaction rates associated with trivalent and hexavalent chromium cannot be explained by differing release from the patch test vehicle.

### 5.6.3.2 Animal Studies

The roles of valence states in chromium hypersensitivity have been extensively investigated in animal studies, which have utilized mainly guinea pigs. As the guinea pig cannot be sensitized epicutaneously by chromium compounds in aqueous solutions, and is sensitizable only inconsistently by this route by chromium in a variety of matrices, special techniques that enhance sensitization are needed in this animal model. The most successful method incorporates the hapten in Freund's complete adjuvant (a water-in-oil emulsion), and involves a combination of intramuscular, intradermal and epicutaneous administration. (Polak, 1983). Thus, in guinea pigs and applying the latter method, Polak et al. (1973) found that trivalent chromium chloride possessed a sensitizing capacity equal to that of hexavalent potassium dichromate, but upon subsequent epicutaneous and intradermal challenging elicited reactions of lesser intensity than the latter compound.

The findings of most investigators concerning chromium contact sensitivity in the guinea pig have been compatible with those of Polak et al. (1973). The observations of Gross et al. (1968) were very similar, and van Neer (1963) found that intradermal application of hexavalent potassium dichromate and of trivalent chromium sulphate induced contact sensitivities of the same degree. However, upon epicutaneous testing, only potassium dichromate yielded positive results; intradermal challenges were not given. Mali et al. (1963) observed that administration of chromium sulphate induced contact sensitivity less frequently and less intensely than hexavalent salts; skin responses to challenges with trivalent chromium were also less pronounced. Polak (1983) notes, however, that Mali et al. utilized lower amounts of elementary chromium equivalents in their experiments than did Polak et al. (1973). Shmunes et al. (1973) reported that guinea pigs sensitized to potassium dichromate reacted more intensely to challenges with this compound than with trivalent chromium chloride.

With the exception of two reports of equivalent inducing and eliciting capacity in guinea pigs (Jansen and Berrens, 1968; Schwarz-Speck, 1968, cited in Polak, 1983), the animal findings are consistent with near equipotency of hexavalent and trivalent compounds in sensitivity induction (by the methods used in guinea pigs), but superior elicitory capacity of hexavalent chromium when applied intradermally or epicutaneously. As already pointed out (Section 5.6.2), permeability differences appear to account for these differential eliciting responses. The clinical and experimental evidence for this is presented in the next section.

### 5.6.4 The Relationship Between Valency and Penetrability

Hexavalent chromium readily traverses the epidermis to the dermis (Mali *et al*., 1964; Czernilewski *et al*., 1965). In contrast, the rate of skin penetration by trivalent chromium has generally been reported to be much lower than that of hexavalent chromium (Mali *et al*., 1964; Spruit and van Neer, 1966). From a chemical viewpoint, the nature and concentration of trivalent chromium salts strongly determine their penetrating capacity (see earlier comments, Section 5.6.2). Samitz *et al*. (1967) have shown in laboratory diffusion experiments that for equivalent concentrations in phosphate buffers in the pH range 5-7, the order of diffusion through human skin was:

chromium(III)nitrate <chromium(III)sulphate <chromium(III) chloride <potassium dichromate. Ion exchange experiments suggested that the diffusivity of the chromium(III) salts declined with increasing positive charge on the active (diffusing) species. Wahlberg and Skog (1965), utilizing 'isotope disappearance measurements' found that for concentrations of 0.017 to 0.239 M, chromium(III) chloride and sodium chromate readily penetrated undamaged skin. However, in higher concentrations (0.261 to 0.398 M), hexavalent sodium chromate penetrated two-fold faster than trivalent chromium chloride.

Liden and Lundberg (1979) studied the histological distribution of hexavalent chromium following penetration of intact human skin _in_ _vivo_. Tangential sections of punch biopsies from patch-tested skin were analyzed for chromium content 5, 24, and 72 h after application of potassium dichromate. Two maxima of chromium concentration were observed: at the dermal-epidermal junction and in the upper mid-dermis. A steady state of skin chromium content was attained by 5 h, with penetration continuing at 72 h.

The local fate of chromium has been studied when it is _administered intracutaneously_ to human subjects (thus by-passing the skin barrier). Doses of sodium chromate and chromium trichloride were given in concentrations required for elicitation of positive test reactions. Ten min following administration of 0.5 µg Cr, only 50% of the sodium chromate remained at the test site, but about 15%

was identifiable after 2 d and 5% after two months. Chromium trichloride disappeared at a much slower rate. Half of the injected chromium (5 μg) disappeared in 5-12 d, with a retention of about 45% of the original dose at the test site after one month (Bang Pedersen et al., 1969; Bang Pedersen and Naversten, 1973). A dose of 0.5 μg Cr administered as Cr(III) chloride disappeared at a rate intermediate between that described for the 0.5 μg chromate and the 5.0 μg of the chloride. Similar results were observed in both allergic and non-allergic subjects. (As an aside, it is noteworthy that the loss of the $^{51}$Cr label from the plasma and erythrocytes of the patients in the case of the chromate injection, and from plasma for the chromium(III) chloride administration, conformed to the patterns described in Sections 3.4.2 and 3.4.3.) Interestingly, in two chromium-sensitive men tested 3 and 4 y earlier by intracutaneous injection with chromium trichloride, Fregert (1971) found it necessary to excise the test sites because of relapsing "flare-up" reactions. Analysis of the excised skin revealed persistence of 21% and 25% of the original injected chromium dose.

Bang Pedersen et al. (1970) have also quantitatively assessed the disposition of sodium chromate applied epicutaneously to two chromate-sensitive patients and two normal controls. After 2 d about 10% of the dose (20.8 μg Cr) was identified at the test site and approximately 90% on the patch itself. That some absorption had occurred was demonstrated by positive reactions in the sensitive subjects. At one month, between 0.6 and 1% of the labelled chromium remained in the skin of control subjects at the test site; in one allergic subject, 0.1% of the original dose persisted and in the other none was identifiable.

Evidently, upon intracutaneous administration, allergic and non-allergic subjects do not differ in their handling of chromium doses. On the other hand, sensitive subjects may have more rapid clearance of epicutaneously administered chromium, perhaps because of desquamation of cells from the inflamed test site. Other research results are also inconsistent with respect to relative chromate absorption in the skin of sensitized and normal subjects (Czernilewski *et al*., 1965; Heise and Mattheus, 1968, cited in Polak, 1983).

### 5.6.5 The Relationship Between Valency and Protein Binding Capacity

Following absorption, hexavalent and trivalent chromium behave differently, with respect to protein binding. It is generally accepted that low-molecular-weight substances, such as chromium compounds, become immunogenic only after conjugation with protein carriers (Landsteiner and Jacobs, 1935; Section 3.6.3). As already documented at some length (Sections 3.3.4.3, 3.3.5, 3.3.6, 3.4.2 and 3.6.2), chromate is readily reduced *in vitro* and *in vivo*, and the resultant Cr(III) ion binds strongly to proteins, polynucleotides and other complexing biomolecules. For example, Gray and Sterling (1950) found, following intravenous injection of dogs with $^{51}$Cr-labelled chromium chloride, that almost the complete dose was bound strongly to plasma proteins, while labelled sodium chromate was associated mainly with erythrocytes. Intracutaneous injection in humans of labelled sodium chromate and chromium(III) chloride gave similar results (Bang Pedersen _et_ _al_., 1969; Bang Pedersen and Naversten, 1973). It is known that erythrocyte chromium derived from chromate uptake is bound to haemoglobin as Cr(III) (Sections 3.3.4.2 and 3.4.2).

As pointed out in Section 3.2.1, chromate is an anion which can act as a complexing agent (ligand) toward metal ions. In contrast, Cr(III) is a metal ion with considerable binding capacity. Not surprisingly therefore, Anderson (1960b) has demonstrated that trivalent chromium reacted more readily with skin proteins than chromate, although the valence state of ultimate binding for the latter was not studied. Similarly, Polak _et_ _al_. (1973) were unable to demonstrate any protein binding of chromate following incubation of guinea pig serum with potassium dichromate. Incubation with chromium(III) chloride resulted in considerable binding to serum proteins. Neither could Magnus (1958) demonstrate any stable binding of chromate to a number of proteins by paper electrophoresis. Samitz and Katz (1964) have demonstrated the reduction of hexavalent potassium dichromate to trivalent chromium by skin proteins and other constituents such as ascorbic acid and lactic acid. As discussed in Sections 3.3.2 and 3.3.4, chromate has demonstrated potential for oxidizing most biological reducing agents at physiological pH values (both low- and high-molecular-weight molecules). Anderson's work (1960b) illustrated the considerable influence of pH on in vitro protein binding of potassium dichromate, with more binding occurring at lower pH levels. Undoubtedly this conjugation followed reduction to trivalent chromium, especially since the oxidizing power of chromates increases with decreasing pH (see Sections 3.2.1 and 3.2.2.4).

### 5.6.6 The Role of Concentration

The influence of concentration of chromium compounds is not as clear-cut as might be supposed, with respect to affecting sensitizing and eliciting potential. Wahlberg and Skog (1965) in isotope disappearance studies (see Section 5.6.3) demonstrated the occurrence of a peak for relative absorption (at 0.26 M) and a plateau for absolute absorption (0.40 M) of sodium chromate. While total absorption certainly does not decrease with increasing concentration, it has been postulated (Jansen and Berrens, 1968) on the basis of clinical and experimental studies that high concentrations of chromium compounds are less allergenic than low concentrations. The low prevalence of chromium sensitivity among chromate production workers is a case in point (Gafafer, 1953). The high frequency of contact sensitivity in the building industry - where the chromium content of materials is generally low, relative to lower prevalences in certain other industrial settings with ostensibly greater chromium exposure - has been cited in support of this postulate (Polak, 1983). It has been suggested that the induction of immunological tolerance and densensitization (which can be produced in animals) could underly such a phenomenon (Polak, 1983; see Section 5.6.13). However, at variance with this hypothesis are examples of workplaces where high levels of exposure are associated with a high incidence of contact sensitivity, such as the apparent high prevalence reported for car assembly workers (Section 5.2.3) and chromeplaters (Section 5.2.11). Further, the independent effect of concentration has not been determined in such observations, after accounting for that of valency, solubility, pH and presence of organic material. Nonetheless, the possible tolerogenic impact of chromium in the workplace could complicate attempts to establish dose-response relationships.

### 5.6.7 The Roles of Solubility, pH and Reducing Agents

It might be expected that given chromium compounds of a particular valency and at a certain level of concentration, their sensitizing and eliciting potential would correlate with water-solubility. Consistent with this clinically observed relationship are the results of investigations in which various chromium compounds were administered to hypersensitive individuals (Zelger, 1964;

Bockendahl, 1954, cited in Polak, 1983). For example, the water-insoluble lead chromate (Section 3.2.2.2) induced skin reactions only in the most sensitive patients.

Wahlberg (1968) has demonstrated a pH-dependence of percutaneous absorption of sodium chromate, with skin penetration in the guinea pig increasing with increasing pH. This effect of pH is mediated largely through its influence on valence state (Burrows, 1978). A consideration of electrode potentials suggests that the chromate ion is stabilized in basic solution because of a loss in oxidizing power (see Sections 3.2.1 and 3.3.6).

The remaining significant determinant of sensitizing and eliciting potential is the presence of organic matter. It exerts its effect by acting as a reducing agent toward hexavalent chromium (e.g. due to the availability of sulphydryl groups), or through an ability to tie up Cr(III) by complex formation, perhaps even inducing its precipitation.

### 5.6.8 In Vitro Indicators of Hypersensitivity

In addition to the patch test, there are a number of in vitro indicators of chromium hypersensitivity, which at present are of limited clinical applicability. These are briefly summarized in this section. The reader is referred to Polak (1983) for a more comprehensive treatment of this topic.

Polak (1983) demonstrated that, upon stimulation with chromium(III) chloride guinea-pig serum conjugates, peritoneal or lymph node lymphocytes from hexavalent chromium-sensitive guinea pigs release an inflammatory factor called the 'skin reactive factor'. Injection of supernatants containing this factor induces local inflammatory reactions in normal guinea pigs. T-effector cells also release a lymphokine on stimulation with antigen called the 'macrophage migration inhibition factor'. Hexavalent and trivalent chromium salts have been found in _in vitro_ studies to inhibit macrophage migration with peritoneal exudates from chromate-sensitive guinea pigs (Polak, 1983) and leukocyte migration with white blood cells from chromium-allergic human subjects (Tio, 1976; Nordlind and Sandberg, 1983). Increased DNA synthesis upon _in vitro_ stimulation by lymphocytes from sensitized individuals comprises the 'lymphocyte transformation test'. Its use appears to be limited to contact sensitivity studies in animals (Polak, 1983; Siegenthaler _et al_., 1983).

### 5.6.9 The Role of Genetic Factors

Relevant to consideration of the pathogenesis of chromium hypersensitivity is reference to the possible role of genetic factors. Polak (1983) demonstrated, in guinea pigs, strain differences in the likelihood of becoming sensitive to potassium dichromate. No evidence was identified concerning the frequency of chromium sensitivity among the relatives of affected human subjects. However, the frequency of histocompatability antigens has been investigated in chromium-sensitive subjects and in normal individuals. These studies found no relationship between chromium sensitivity and HLA typing (Roupe et al., 1979; Kapoor-Pillarisetti et al., 1981).

### 5.6.10 The Role of Noncutaneous Absorption in Cutaneous Hypersensitivity

Study of chromium absorption by noncutaneous routes has shed light on the mechanisms involved in chromium hypersensitivity of the skin, especially in its chronic forms. Orally administered trivalent chromium is only minimally absorbed through the gut ( ~ 0.5%), while intestinal absorption of hexavalent chromium occurs to a greater degree ( ~ 10%; Donaldson and Barreras, 1966; see Section 3.4.1). It is known that gastric acidity promotes the conversion of ingested chromate to the trivalent state, thus reducing absorption. (No association has been noted between achlorhydria and chromium dermatitis!; Burrows, 1978.)

A number of studies have dramatically shown the effects of oral absorption of even small amounts of chromium in sensitive individuals. Within 2 h of oral administration of 50 μg of potassium dichromate, five chromium-allergic subjects developed acute vesiculation of the palms (Fregert, 1965, cited in Cronin, 1980). Kaaber and Veien (1977) compared the effects in 31 chromium-sensitive patients with current dermatitis, of orally administered potassium dichromate and placebo in a double blind study. Chromate produced exacerbation of the dermatitis in 11 individuals, while placebo was associated with aggravation of the skin condition of two subjects. The dose given was equivalent to 2.5 mg of chromium, regarded as within the range of ordinary daily intake in Danish diets. Goitre _et al_. (1982), using this oral chromium dose, produced exacerbation of chromium contact dermatitis in a construction worker.

No evidence is available concerning the benefits of reduction of dietary chromium in sensitive individuals. Such a strategy would seem difficult to carry out, given the ubiquitous occurrence of chromium in foods (usually in very small amounts).

Chrome-containing metal implants constitute a possible source of exposure which is presently of uncertain significance with respect to induction or elicitation of sensitivity (Carlsson _et al_., 1980).

Little information is available concerning the relationship between inhalational exposure to chromium and hypersensitivity manifestations in the skin. Shelley (1964) reported that inhalation of welding fumes by a chromium-sensitive individual resulted in repeated episodes of hand dermatitis. In another report, exposure to welding fumes was associated with urticaria and asthma in a railway worker. However, the allergen was not identified (Kaplan and Zeligman, 1963).

### 5.6.11 The Role of Interaction with Light

In some individuals light may interact with chromium in the production of hypersensitivity phenomena. Feuerman (1971) reported that Israeli housewives sensitive to chromates in detergents were at increased risk of developing dermatitis upon exposure to sunlight, relative to other dermatological patients without chromium allergy. Wahlberg and Wennersten (1977) demonstrated that short wave ultra-violet light exacerbated the reactivity to chromium in 12 out of 25 chromium-allergic patients. Hannuksela _et al_. (1981) observed a statistically significant increase in prevalence of delayed contact hypersensitivity to chromium among two groups of patients with photodermatitis (14 and 110 subjects, respectively), than in 1,714 patients with other kinds of dermatitis.

### 5.6.12 Concomitant Sensitivities: Independent or Interdependent?

It remains unresolved whether positive reactions to nickel and/or cobalt occasionally observed in chromium-sensitive patients (Fregert and Rorsman, 1966; Cronin, 1980; also see Table 4) are attributable to cross-sensitivity or to independently, simultaneously occurring sensitivity to these metals. In cement dermatitis, concomitant allergy to cobalt apparently occurs only when traces of cobalt are present in the cement (Polak, 1983). Thus, sensitization to cobalt and chromium may be taking place independently. Similarly, it is believed that concurrent exposure to both nickel and chromium compounds may account for the occurrence of positive patch tests to both metals simultaneously. However, sound research is lacking concerning possible cross-sensitivity in chromium allergy.

### 5.6.13 Desensitization, Tolerance and Flare

Lastly, the phenomena of desensitization, tolerance, and flare reactions in chromium hypersensitivity are briefly considered. First, the meaning of these terms in immunological usage is outlined. Loss of the immune response to an antigen or hapten to which an individual has already been sensitized is 'desensitization'. In contrast, 'tolerance' to the development of contact sensitivity can occur in non-sensitized (naive) subjects on exposure to the hapten. And finally, 'flare reactions' may occur at healed sites of previous eczematous lesions or patch tests when the hapten is applied orally or intravenously.

Attempts have been made to desensitize permanently patients with cement dermatitis, by repeated epicutaneous administration of potassium dichromate (Pautrizel et al., 1962; Sourreil et al., 1964a,b). These attempts met with variable success. As noted above, (Section 5.6.10) oral administration of small amounts of potassium dichromate to chromium-sensitive patients produced exacerbation of previous inflammatory sites rather than desensitization (Kaaber and Veien, 1977). The failure to demonstrate desensitization consistently in human subjects is not surprising, given the results observed by Polak (1983) in studies with chromate-sensitized guinea pigs. Intravenous injection of doses of potassium dichromate of the order of the $LD_{50}$, accompanied by an epicutaneous challenge with the same compound within 24 h, was required to induce permanent, complete desensitization. Lower intravenous doses or a delay in the associated epicutaneous application resulted in a temporary desensitization response only. The role of desensitization in humans chronically exposed to chromium, such as in the workplace, is not known. On the other hand, Polak (1983) readily induced complete, permanent hapten-specific tolerance at much lower doses than were required for permanent desensitization. The possible extent of tolerance among workers exposed to chromium compounds is also unknown.

Polak (1983) also extensively investigated the phenomenon of flare-up in guinea pigs, after intravenous injection of the hapten. Flare-up reactions were elicited in chromate-sensitized animals after injection of either potassium dichromate or chromium(III) sulphate. It was suggested that the mechanism involved was related to the Arthus phenomenon. The use of oral administration of potassium dichromate has been suggested for diagnostic purposes, to induce a flare at previously negative skin challenge sites and thus to reveal a "latent state" of chromium hypersensitivity (Schleiff, 1968, cited in Polak, 1983). This method has not been further evaluated.

### 5.6.14 Working Hypothesis of Pathogenesis

A working hypothesis of chromium immunogenicity which unites the diverse observations reviewed in Sections 5.6.3 to 5.6.8 is as follows (Polak, 1983). Chromium in its hexavalent form readily penetrates the skin, whereas most trivalent chromium compounds traverse the epidermis relatively poorly, being readily complexed by skin proteins. Absorbed trivalent chromium tends to bind immunologically irrelevent proteins, and cross cell membranes poorly. Hexavalent chromium, on the other hand, is absorbed in greater concentrations, attains key membrane and intracellular sites, and undergoes reduction to the trivalent state which forms conjugates with proteins, constituting complete antigens. Consistent with this hypothesis are reports that circulating antibodies exist in sensitized animals against trivalent chromium but not against hexavalent chromium (Cohen, 1962; Mali _et_ _al_., 1963; Novey _et_ _al_., 1983), although such results are to be viewed with caution because of the inherent uncertainties and complexities in antibody characterization.

Antibodies do not play a primary role in chromium dermatitis which is mediated principally by peripheral lymphocytes (T-cells). A Cr(III)-protein complex is most likely the form that is recognized by antigen-inexperienced T-cells. Macrophage-like Langerhans' cells are thought to participate in the formation of the antigenic complex, and appear important in both the induction and elicitation phases of contact sensitivity (see Section 3.6.3). Recent work by Siegenthaler _et_ _al_. (1983) confirms that a common antigenic determinant is likely to be involved in chromium contact sensitivity, rather than independent entities corresponding to each valency state. As in earlier experiments (Section 5.6.3.2), guinea pigs sensitized with either trivalent chromium chloride or hexavalent potassium dichromate reacted *in vivo* and *in vitro* to both chromium salts. Reactivity to chromium in both valence states was retained after repeated restimulations with chromium of either valency. Thus a common antigenic determinant is probable, and Siegenthaler *et al*. argue that this determinant is formed by chromium in the trivalent form.

From this model it is clear that the influences of many factors converge to yield clinically observable hypersensitivity manifestations. The nature of some of these manifestations will be strongly dependent on the clinical or laboratory manoeuvre performed to demonstrate them. Thus, the clinical signs may vary with route of antigen administration (Epstein, 1966), the test compound selected, the dose, or the type and composition (e.g. presence of emulsifiers; Rudzki *et al*., 1976) of the vehicle used (Skog and Wahlberg, 1969).

## 5.7 Occupational Asthma

In this section, evidence is reviewed concerning effects of occupational chromium exposure on reversible bronchoconstrictive disease, with a focus on hypersensitivity responses. A discussion of nonreversible airways disease and other pulmonary diseases is provided in

Chapter 6.

Given the well-documented irritant and sensitizing properties of chromium compounds, it is not surprising to encounter literature evidence for chromium - associated, occupational bronchial hypersensitivity. This evidence is however limited in extent, deriving predominantly from a number of case reports and case series studies. Despite the relative uncommonness of reports of an association of chromium exposure with bronchial asthma, the etiologic relation is strongly supported in most instances by consistent chronological patterns with regard to exposure, and by confirmatory responses to bronchodilator therapy. In these reports, the characteristic combination of (apparent) rarity, response to provocation, and reversibility (spontaneous or by treatment) suggest a hypersensitivity mechanism (Pepys, 1977).

Both immediate (onset in minutes) and nonimmediate (onset in hours) asthmatic responses (Pepys, 1977) to chromium compounds are known. Williams (1969; 1973) has reviewed and summarized the various occurrences reported up to 1969. Isolated cases of asthma from chromium compounds have been observed for the following occupational exposures: to dichromates, chromic acid, chromite ore, chromate paint pigments, and welding fumes. A few specific examples will illustrate these case reports. Card (1935) precipitated asthmatic attacks in a chromium plater/polisher 2 1/2 - 5 h after the intradermal injection of 0.004g potassium dichromate or 0.004g potassium chromate. A few years earlier, Joules (1932) had observed a positive response within 1 h in a similar provocation in a metal plater. Marechal (1957) has reported 4 cases of asthma in association with exposure to chrome yellow, which contained lead and zinc chromates. A remaining report of chromium-associated asthma that deserves special mention concerns the response to chromic acid by ferrochromium workers (Broch, 1949; Lillingston, 1950). Two patients had violent asthmatic attacks to fumes from the factory furnace. Skin tests revealed that chromic acid was the offending substance.

In a non-atopic electroplating worker with a clinical picture consistent with occupational asthma, Novey et al. (1983) observed, on inhalation challenge by chromium sulphate, clearcut depression of $FEV_1$ within 15 minutes of cessation of the challenge, with a return to prechallenge levels in 90 minutes. Interestingly, challenge with a nickel sulphate solution in the same patient produced a biphasic $FEV_1$ response, with the second depression maximal at 4 h. Serologic studies provided some evidence for circulating IgE antibodies to both chromium and nickel in this patient, although control tests for non-specific contributions in the radioimmunoassays appear to have been inadequate.

Another recent report of detailed clinical assessment of chromium-associated asthma is that of Keskinen et al.

(1980). A total of 5 stainless steel workers with work-related bronchospastic symptoms were documented, of which 2 were observed over a 2-y period of non-exposure. Challenge by manual stainless steel welding yielded significant bronchial obstructive responses, whereas metal-inert gas welding and manual welding of mild steel were negative. This response was blocked by pretreatment with both disodium cromoglycate and with beclomethasone, but not with placebo. The main distinction in type of exposure between stainless steel and mild steel welding is that mild steel does not have chromium and nickel as constituents. A comparison of the composition of fumes generated by manual and inert gas welding of stainless steel has shown that manual welding generates considerably higher levels of water-soluble chromium(VI) (Stern, 1982; see Section 4.4.2.3).

The most comprehensive study of the role of chemicals (including chromate) in bronchial asthma is that of Popa _et al_. (1969), who studied 48 subjects with clear historical evidence of asthma occurrence in association with exposure to micromolecular substances having both irritating and sensitizing properties. Nine of these subjects had occupational exposure to dichromate (6 tanners, 1 cement worker, 1 pottery worker, and 1 unclassified); however all but 2 were also exposed to other possible respiratory sensitizers. In four of the chromate-exposed individuals, the inhalation challenge was positive. These workers were considered to have a nonspecific bronchial hyperreactivity, because of the failure to demonstrate sensitization by immunological tests. The diagnosis for the fifth worker was delayed hypersensitivity to chromate, for both the asthmatic and cutaneous responses. The remaining four patients could not be classified readily. They exhibited some but not all the features of allergic asthma. In addition to the person with the delayed responses, some of the other workers also exhibited delayed cutaneous sensitivity. Interestingly, concomitant contact sensitivity to chromate was also noted in some of the case reports cited above (Williams, 1969; Keskinen et al., 1980).

Two additional epidemiological studies pertain to bronchial chromate hypersensitivity. Both were cross-sectional in design, and only utilized questionnaires to ascertain asthma. Among chromium platers, Royle (1975) found that 13.1%, compared to 9.8% of controls ($p < 0.025$), reported a history of symptoms compatible with asthma. While Langård (1980) observed a greater prevalence of wheeze and dyspnea among ferrochromium workers than among controls, the latter group were considerably younger than the exposed workers. The prevalence among the ferrochromium group was somewhat lower than that observed among ferrosilicon and maintenance workers in the same plant. Langård's survey, perhaps in part because of methodological limitations, gives no indication that chromium bronchial hypersensitivity is more frequent among ferrochromium workers than the uncommon case reports suggest.

Finally, it should be noted that there is no evidence whatsoever in the literature to allow estimation of doses required for induction or elicitation of chromate-induced occupational asthma.

## 5.8 Summary

Chromium is the second most common skin allergen in the general population, after nickel. In men it is the most frequent sensitizer. The prevalence of chromium dermatitis is the highest in construction workers using cement. Other occupational exposures associated with chromium sensitivity are numerous, of which chromium plating, tanning of leather, application of anti-corrosive agents, and printing are representative industrial activities. There is little evidence that the prevalence of chromium dermatitis, correlates with dose. The consensus is that the outlook for persons with chromium dermatitis is bleak. Avoidance strategies, including replacement of chromium-containing constituents and addition of suppressors, show promise and have been implemented in a few instances. Although respiratory sensitivity to chromium compounds is known, it occurs infrequently.

In terms of pathogenesis, chromium contact sensitivity of the skin is a characteristic cell-mediated phenomenon (delayed hypersensitivity). Hexavalent chromium compounds generally are more potent inducers and elicitors of sensitivity than trivalent compounds; however, the Cr(III) ion very likely figures in the formation of the ultimate and common antigenic determinant. Concentration, solubility, pH and presence/absence of reducing agents are significant influences on the likelihood and intensity of sensitivity associated with contact exposure to chromium compounds. There is no clear evidence that chromium hypersensitivity is strongly genetically determined in humans. Oral ingestion of chromium can lead to skin reactions in sensitive subjects, but the overall significance of non-cutaneous absorption of chromium is uncertain with respect to induction and elicitation of sensitivity and production of tolerance. Sensitivity to light is a potentiating factor in some subjects. The existence of cross-sensitivity to other salts has not been excluded, but many "double allergies" could plausibly derive from the development of separate, independent sensitivity states. Animal studies would suggest that the prospects for desensitizing interventions in humans are poor. It is not known whether chronic exposure to chromium results in desensitization in man. The induction of tolerance may be taking place to an uncertain extent in the community and in workplaces, and has been postulated to be a dose-related phenomenon.

## Table 5.1

Prevalence of Positive Patch Tests to Potassium Dichromate Among Patients Investigated for Contact Dermatitis, By Sex

| | | % chromate positive | | |
|---|---|---|---|---|
| Setting | N | M | F | Reference |
| Belgium | 400 | 17.3 | 9.7 | Lachapelle and Tennstedt, 1979 |
| Brazil | 536 | 25.5 | 5.7 | Moriearty *et al.*, 1978 |
| 'Europe' | 4825 | 10.7 | 3.6 | Fregert *et al.*, 1969 |
| 'North America' | 1200 | 10.0 | 6.0 | North American Contact Dermatitis Group, 1973 |
| 'Scandinavia' | 5558 | 11.7 | 3.0 | Magnusson *et al.*, 1968 |
| Scotland | 1312 | 7.0 | 4.0 | Husain, 1977 |

Table 5.2

Prevalence of Positive Patch Tests to Potassium Dichromate Among Patients Investigated for Contact Dermatitis, North America (men and women combined)[a]

| City | N | % chromate positive |
|---|---|---|
| Bangor | 59 | 10 |
| Detroit | 20 | 20 |
| Hanover | 197 | 9 |
| Marshfield | 129 | 2 |
| New Orleans | 24 | 12 |
| New York | 44 | 9 |
| Portland | 229 | 10 |
| Richmond | 207 | 4 |
| San Francisco | 126 | 9 |
| Vancouver | 165 | 8 |

a. Based on data reported by North America Contact Dermatitis Group, 1973.

## Table 5.3

### Age Ranges of Chromate-Sensitive Subjects, St. John's Hospital for Diseases of the Skin, 1967-1976, by Sex

| Age Range | Men | Women |
|---|---|---|
| under 21 | 15 | 13 |
| 21 - 30 | 61 | 35 |
| 31 - 40 | 122 | 23 |
| 41 - 50 | 125 | 29 |
| 51 - 60 | 121 | 41 |
| 61 - 70 | 65 | 15 |
| over 70 | 4 | 6 |

a. Reproduced in modified form from Cronin (1980).

Table 5.4

Associated Sensitivity to Cobalt and/or Nickel
Among Chromate-Sensitive Subjects,
St. John's Hospital for Diseases of the Skin, 1973-1976[a,b]

| | N | | | |
|---|---|---|---|---|
| Sensitivity | Men | (%) | Women | (%) |
| $Cr_6^+$ $Co^-$ $Ni^-$ | 143 | (70) | 47 | (57) |
| $Cr_6^+$ $Co^+$ $Ni^-$ | 38 | (19) | 7 | (9) |
| $Cr_6^+$ $Co^-$ $Ni^+$ | 10 | (5) | 9 | (11) |
| $Cr_6^+$ $Co^+$ $Ni^+$ | 12 | (6) | 19 | (23) |
| Total $Cr_6^+$ | 203 | | 82 | |

a. Reproduced from Cronin (1980).

b. $Cr_6$ denotes Cr(VI); superscript +, positive reaction; superscript -, negative reaction.

Table 5.5

The Ten Most Important Examples of Eczematogenic Exposures to Chromium Compounds[a]

| Chromium containing materials or objects | Profession or places of contact | Chromium compounds responsible |
|---|---|---|
| Chromium ore | Industrial chromium production | Chromite |
| Chrome baths | Electro-plating industry | Chromic acid<br>Sodium dichromate |
|  | Graphic trade | Chromates |
|  | Metal industry | Chromates<br>Zinc chromate |
| Chrome colors and dyes | Painters and decorators, graphic trades, textile, rubber, glass and china industries | Chromic oxide green, chromic hydroxide green, chrome yellow (lead chromate) |
| Lubricating oils and greases | Metal industry | Chromic oxide |
| Anticorrosive agents in water system | Diesel locomotive workshops and sheds, central heating and air-conditioning systems | Alkali dichromates |
| Wood preservation (Wolman salts) | Wood impregnation, furniture industry, carpenters, miners | Alkali dichromates |
| Cement, cement products, quick-hardening agents for cement (e.g., Sika 1) | Cement production, manufacture of cement products, building trades | Chromates |
| Cleaning materials (Eau de Javelle), washing and bleaching materials | Housewives, cleaners, laundry workers | Chromates |
| Textiles, furs | Textile and fur industries, everyday life | Chromates |
| Leather and artificial leather tanned with chromium | Leather and footwear industry, everyday life | Chromium sulfate and chromium alum |

a. Reproduced in modified form from Polak (1983).

Table 5.6

Threshold Concentrations for Positive Skin Tests In Chromium-Hypersensitive Patients[a]

| Agent | Valency | Concentration (%) | Number of Positive Patients[b] |
|---|---|---|---|
| $K_2CrO_4$ | VI | 0.001 | 3 |
| | | 0.005 | 9 |
| | | 0.01 | 10 |
| | | 0.05 | 11 |
| $H_2CrO_4$ | VI | 0.001 | 1 |
| | | 0.005 | 5 |
| | | 0.01 | 2 |
| | | 0.05 | 5 |
| $K_2Cr_2O_7$ | VI | 0.01 | 2 |
| | | 0.05 | 17 |
| | | 0.1 | 14 |
| $KCr(SO_4)_2$ | III | 0.05 | 3 |
| | | 0.1 | 7 |
| | | 0.5 | 12 |
| | | Negative | 6 |
| $Cr(NO_3)_3$ | III | 0.05 | 3 |
| | | 0.1 | 2 |
| | | 0.5 | 5 |
| | | Negative | 18 |

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a. Reproduced in modified form from Polak (1983); also see Zelger (1964).

b. Number of patients tested were ?

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# 6. OTHER HEALTH EFFECTS OF CHROMIUM

## 6.1 Overview

The risk of contracting cancer or developing hypersensitivity of the skin or respiratory system on exposure to chromium compounds has been reviewed in Chapters 4 and 5, respectively. Other debilitating effects associated with excessive exposure to chromium compounds have also been well documented in the literature, some of which are indeed more common than those mentioned (NIOSH, 1973, 1975). For example, skin ulcerations are the most common lesions in the occupational setting, and are experienced by tanners, electroplaters and dichromate workers (Burrows, 1983). It is also well known that chromium(VI) compounds may cause acute, subacute or chronic injuries to the nasal tissues of exposed workers, resulting in mucosal irritation, ulceration and even nasal septal perforation (Bidstrup, 1983). As will be reviewed briefly below, other non-allergic, inflammatory effects on the respiratory system that have been reported in association with chromium exposure include: chronic rhinitis, chronic sinusitis, laryngitis, bronchitis, acute chemical pneumonitis, and even a suggestion of chromitotic pneumoconiosis. Conjunctivitis has also been attributed to chromium exposure, as well as olfactory disorders and dental abnormalities. In addition, isolated reports exist of inflammatory and ulcerative conditions of the gastrointestinal tract, and of liver and kidney damage. And finally, there has been some concern expressed that chromium may have a deleterious effect on reproduction, possibly causing reduced fertility and birth defects. This, too, will be reviewed in this concluding chapter.

## 6.2 Irritant Effects of Chromium on Skin and Nasal Tissues

### 6.2.1 Description and Prevalence

Ulcerations of the skin and nasal septum in workers exposed to chromium(VI) compounds have been recognized for well over a century. As early as 1827 Cumin described chrome ulcerous lesions of the skin and in 1863 Bécourt and Chevallier reported ulcerous lesions of both skin and nasal tissues. NIOSH (1975) has reviewed the dozens of reports in the literature in this area and has concluded: "It may be stated unequivocally that chromium(VI) may cause skin ulcers, ulcers of the nasal mucosa and perforations of the nasal septum". Moreover, it is pointed out that chrome ulcers may appear anywhere on skin given sufficient contact with acidic or alkaline solution of hexavalent chromium. The most frequently reported sites are the finger nail root areas, skin folds over knuckles, finger webs, dorsum of hands and forearms, since skin abrasions are more prone to occur at these locations and broken skin strongly facilitates chrome ulceration (Edmundson, 1951; Maloof, 1955). Scars remain after healing. Maloof (1955) described these lesions as beginning as "painless papules or vesicles that eventually ulcerate and penetrate deeply into the tissue." He attributed the poor healing of the ulcers to tenacious deposits of chromium(III) with prolonged eroding action. Topical applications often include EDTA to aid both the reduction of hexavalent chromium to the trivalent form and the removal of the latter by chelation (Maloof, 1955; Burrows, 1983).

An annotation in the British Medical Journal twenty years ago (Anon, 1963) succinctly summarizes years of clinical experience with chrome ulceration of the nasal septum. Four well-defined stages are recognized (also see Bidstrup, 1983), progressing from nasal irritation, to ulceration and perforation of the septum. Initially, redness and irritation of the mucosa covering the septum occur, which are accompanied by rhinorrhea and followed by blanching and the development of adherent crusts. Nose-picking invites further contamination by chromates. After an interval that ranges from days to weeks, ulceration occurs. Subsequently, the septal cartilage becomes eroded, leaving a perforation of the septum. The perforation often increases in size, but neither the bony part of the septum nor the anterior and lower parts become involved. The shape of the nose is not affected. During the stage of active ulceration, slight bleeding is frequent. Commonly, within about three months the edges of the perforation are healed, providing it is not aggravated by picking. Complications are rare and the condition does not give rise to disability. There is no evidence that malignant change occurs at the site of either nasal or skin ulceration caused by chromium(VI) compounds, and a loss of sense of smell appears to be infrequent. It has also been noted that there is no apparent relationship between the development of chrome ulcers and the prevalence of sensitization, as indicated in Section 5.3.11.

Given the irritant and corrosive nature of soluble, hexavalent chromium, it is not surprising that not only direct cutaneous contact but also inhalation of dust or mist may inflict damage. Chrome ulceration of the nasal septum may occur in any industry in which it is possible for hexavalent chromium to be deposited in this region of the nose. Thus "chrome plating, chrome tanning, dyeing and finishing, and the production of bichromates" were identified as industries in which this is probable (Anon, 1963). Chromate dust (including pigments), dichromate dust, or chromic acid mist appear responsible. As Langård and Norseth (1979) note, in the case of tannery workers, the skin ulcers may be due to chromium(III) compounds (Maloof, 1955). However, as pointed out by Stern (1982), chromates are also common in tanneries (as starting materials or in the 'two bath' tanning process).

Indeed, the irritant effects of chromate on skin and nasal tissue have been reported in many countries for chromium platers (e.g., Kleinfeld and Rosso, 1965; Gomes, 1972; Henning, 1972; NIOSH, 1973, 1975; Royle 1975 a,b;

Horiguchi, 1980); among chrome strippers (e.g., Mitchell, 1969); for galvanization shop employees (Petrzela et al., 1981); in individuals exposed to zinc chromate pigment (Langård and Norseth, 1979); in ferrochromium workers (Axelsson et al., 1980; Langård et al., 1980); and especially among chromate production workers (Edmundson, 1951; Mancuso, 1951; Gafafer, 1953; NIOSH, 1973, 1975; Krishna et al., 1976; Hayes et al., 1979; Watanabe and Fukuchi, 1981). Claims that skin and nasal ulcerations are associated with trivalent chromium exposure (Mancuso, 1951) have not been substantiated. As explained in Section 4.4.1.1, the plant studied by Mancuso was characterized by mixed exposure to both hexavalent and trivalent chromium.

The reported prevalence of skin and nasal ulcerations among workers exposed to hexavalent chromium is high. Among chromate production workers, a prevalence of nasal septal perforation near 60% has been consistently reported (Edmundson, 1951; Mancuso, 1951; Gafafer, 1953). Edmundson (1951) found that 70% of the workers examined had chrome skin ulcers, and 47% were inflicted with both the skin and nasal lesions. Occurrence among chromeplaters was also substantial. In a study of 997 platers by Royle (1975b), the results of a questionnaire indicated that 17% of the workers had nose bleeding, 13% had nasal ulcers, 5% had nasal perforations, and 22% had skin ulcers. These percentages increased in a subgroup of 369 workers, who had been exposed for more than 5 y, to 16, 17, 9 and 32%

respectively. In Britain, both skin and nasal septum ulcerations are notifiable. Although the number of notifications of skin ulcers have been declining since 1965, as many as 50 - 100 are still reported annually (Burrows, 1983). The majority of cases occur in the chromium plating industry (Royle, 1975a).

### 6.2.2 Threshold Chromate Concentrations

As Langård and Norseth (1979) have concluded, the prevalence of chromium skin ulcers bears no relationship to atmospheric chromium concentrations; presumably because the development of these lesions depends on local deposition of the chromium compound on the skin and on the presence of any pre-existing cutaneous lesions. Although most investigators stress the importance of individual work practices in preventing skin and nasal mucosal lesions (e.g., Anon, 1963; Bidstrup, 1983), it is acknowledged that the only effective way to prevent formation of nasal ulcers is to limit chromic acid mist from entering the workplace atmosphere (Henning, 1972). Methods of exhaust ventilation and spray suppressants, as well as of personal protection, are outlined in detail by Henning (1972) and Mitchell (1969).

Many of the investigators reporting chrome ulcers in association with exposure to mists of hexavalent chromium from plating tanks have provided atmospheric levels (Bloomfield, 1928; Kleinfeld and Rosso, 1965; Mitchell, 1972; Gomes, 1972; a number of studies cited in NAS, 1974 and NIOSH, 1975; Royle, 1975b). For example, Gomes (1972) reported that 86% of the workers examined had lesions due to chromic acid exposure, but noted that the T.L.V. of 0.05 mg Cr $m^{-3}$ was exceeded 50% of the time and extreme levels as high as 0.7 mg Cr $m^{-3}$ had been noted periodically. At the mean chromium(VI) exposure levels listed in Table 4.2B (typically, 0.03 - 0.30 mg Cr $m^{-3}$), Mancuso (1951) and Gafafer (1953) found the prevalence of skin and nasal septum ulcerations to be very high among chromate production workers, as already indicated.

Chromate-induced perforations of the nasal septum have often been considered to occur only when the atmospheric concentrations of chromic acid exceed 0.05 mg Cr $m^{-3}$ (Bloomfield 1928; NIOSH, 1975). This conclusion does not seem to be supported by more recent data. For example, Royle (1975b) reported that the risk of skin and intranasal ulcerations increased progressively with the duration of chromic acid exposure, which was shown in a single personal monitoring survey to be less than 0.015 mg Cr $m^{-3}$ in 40 of 42 plants examined. Based on a questionnaire, he found that 22% of the platers had had skin ulcers, but when the duration of exposure exceeded 5 y, the incidence increased to 32%. Royle's results suggest that chromic acid at 0.015 mg Cr $m^{-3}$ was sufficient to cause symptomatic nasal ulcerations. Lucas and Kramkowski (1975) have found ulcerations at average levels of 0.002 mg Cr $m^{-3}$ (range 0.001 - 0.010). Comparable findings are reviewed in NAS

(1974). Mitchell's (1969) data suggest that a reduction of chromate exposure levels to 0.005 mg Cr $m^{-3}$, obtained by the installation of exhaust ventilation of chrome plating tanks (also see Kleinfeld and Rosso, 1965; NIOSH, 1975), prevented the occurrence of previously observed nasal lesions. In addition to chromic acid exposure, it is important to emphasize that settled, chromate-rich dust appears to be ubiquitous in electroplating plants, even when air levels are low (Royle, 1975b). This additional source of chromium(VI) may well contribute to increased skin contact. And finally, Langård and Norseth (1979) observed that while they had noted septal ulceration after 2 weeks and perforation after 2 months of exposure to zinc chromate dust at ~0.3 mg Cr $m^{-3}$, they did not observe ulcerations or perforations in workers exposed to the same dust at 0.006 - 0.03 mg Cr $m^{-3}$ for up to 18 months.

Within a retrospective mortality study of chromate production workers, Hayes et al. (1979) investigated by case-control pairs classified by the presence or absence of a history of lung cancer, the possible association of lung cancer with skin ulcers, nasal perforation and dermatitis. Only a history of dermatitis was associated with lung cancer with a relative risk of 3.0 ($p < 0.05$). However, the occurrence of all 3 conditions within an individual was also highly associated with lung cancer history (relative risk of 6, $p < 0.05$). Hayes et al. (1979) expressed the view that this association between lung cancer and chromate-related morbid conditions likely indicates that greater exposure implies greater risk. However, they conclude: "Our knowledge of the epidemiology of these morbid conditions is, however, too scant to use them as direct indicators of cancer risk or as determinants for maximum allowable concentrations of carcinogens".

## 6.3 Other Irritant and Miscellaneous Findings

In the extensive medical survey by Gafafer (1953) of chromate production workers, a number of other chromate-associated health effects were revealed. There was an excess of severe red throat, a yellowish discolouring of the teeth and tongue, and a higher percentage of workers with gingivitis and periodontitis (also see Gomes, 1972). Many of the inflammatory responses observed by Mancuso (1951) were not found (chronic chemical rhinitis, sinusitis and pharyngitis, laryngitis with hoarseness). Gastric distress (Mancuso, 1951; Lucas and Kramkowski, 1975), conjunctivitis (Gafafer, 1953; Gomes, 1972), and olfactory sense impairment (Mancuso, 1951; Watanabe and Fukuchi, 1981) have also been reported for workers exposed to hexavalent chromium.

## 6.4 The Role of Chromium in Bronchitis, Pneumonitis and Pneumoconiotic Processes

Occurrence of chromium-associated bronchitis is not well documented. Bronchitic symptoms have been reported for workers using alkaline dichromates (see Williams, 1969) and chromate production workers (Capodaglio *et al.*, 1975;

Wieser et al., 1982). Evidence suggestive of bronchitis for electroplaters was provided by Royle (1975b), although assessment was by questionnaire only. Alderson et al. (1981) observed a total of 39 deaths associated with chronic bronchitis in chromate workers, which did not differ significantly from the number expected, although there was an excess ($p < 0.001$) in one of the three factories examined.

In 1939, Letterer described a "chromium-silicotic" lung in a sandblaster in a ferrochromium department. The diffuse enlargement of the alveolar septa and the chronic interstitial and alveolar pneumonia observed were believed by the author to be caused by inhalation of chrome dust. This finding was consistent with a previous report by Lukanin (1930) of pulmonary fibrosis in a chromate worker. Machle and Gregorius (1948) suggested the existence of a chromate-induced chemical pneumonitis from their survey of workers in chromate-producing plants, and Meyers (1950) reported two cases of acute chromic acid poisoning which resulted in severe and prolonged pulmonary disease. Mancuso (1951) detailed the case of a worker with chemical pneumonitis which was attributed to his work at a chromate plant. He and his colleagues also described several cases of spotty, moderately severe, but not nodular, pneumoconiosis which they called chromitotic pneumoconiosis (Mancuso, 1951; Mancuso and Hueper, 1951).

Sluis-Cremer and Du Toit (1968) have reported fine nodular pneumoconiosis in a few chromite miners in South Africa. They described the nodulation as finer in quality, though somewhat more radio-opaque, than in simple coalminers' pneumoconiosis. However, these authors describe animal experiments which showed that chromite injected intratracheally into rats initially produced an acute inflammatory reaction, but that chromite deposits which accumulated and remained in the lungs were not fibrogenic. They also noted the study by Worth and Schiller (1955), in which intraperitoneal chromite caused an initial, mild inflammatory reaction in mice, but produced no long-term sequelae. They therefore concluded that pneumoconiosis in chromite miners is due to the deposition of radio-opaque chromite dust in the tissues, but that the condition is benign and causes no fibrosis. Reports of pneumoconiosis in chromite miners appeared in the U.S.S.R. as well (e.g., Andrievskaya and Mislavskaya, 1949).

Princi _et_ _al_. (1962) have described a pulmonary disease of progressive fibrosis characterized clinically by recurring bouts of acute pneumonitis, ventilatory impairment, as well as linear and nodular fibrosis in the chest x-ray. These pulmonary abnormalities were attributed to exposure to high concentrations of metallic (including chromium) silicide fumes. These authors suggested that this disease had occurred in workers of other ferroalloy plants, although it had not been previously recognized.

Average air levels of total dust in the plant investigated were 15 ± 45 mg $m^{-3}$ with the quartz content below 2%. The pathogenesis of this condition, which has since been noted by other investigators (Davies, 1974), is not yet clear.

The role of chromium in the progressive fibrosis described by Princi _et al_. (1962) has not been established, especially since the levels of total dust exposure were very high. For some of the other cases described above, either trivalent or hexavalent chromium or both might have been determinants in the chromium-associated pneumoconiosis described (Langård and Norseth, 1979). However, the clinical significance of this is again unclear.

Finally, Bovet _et al_. (1977) investigated the spirometric alterations in chromate-exposed workers in the electroplating industry. They found that urinary chromium concentrations significantly correlated with reductions in $FEV_1$ (forced expiratory volume over one second) and $FEF_{25-75}$ (forced expiratory flow evaluated at 25-75% expiration of the vital capacity), but not with VC (forced vital capacity). Capodaglio _et al_. (1975) also demonstrated decrements in respiratory function among workers producing dichromates and chromic acid. By contrast, median vital capacity readings for chromate and non-chromate production workers showed practically no difference (Gafafer, 1953). The results obtained by Langård (1980) for ferrochromium workers strongly suggest that the high level of total dust, rather than chromium(III) and chromium(VI) compounds _per se_, was responsible for the generalized obstructive lung disease observed (also see Section 5.7). Examination of welders has also shown that there is a greater prevalence of small airway disease among shipyard mild steel MMA welders (no chromium exposure) than among stainless steel MMA welders [with exposure to chromium(VI)] (Kalliomäki _et al_., 1982).

In conclusion, chromium exposure is associated with a moderate risk of pulmonary hypersensitivity (Section 5.7), and heavy exposure to chromic acid may result in acute chemical pneumonitis. Otherwise, there is little evidence to suggest that long-term exposure to chromium compounds result in non-malignant, chronic pulmonary disease.

## 6.5 Gastrointestinal, Hepatic and Renal Impairments

Mancuso (1951) conducted a gastrointestinal X-ray examination of about 100 chromate workers. The findings (10% had evidence of ulcer formation and 6% of hypertrophic gastritis --- compared to 5% in both categories in a control group of cement workers) indicated a highly probable relationship between occupational exposure and gastrointestinal pathology. Lucas and Kramkowski (1975) also reported that 5 out of 11 "hard" chromium electroplaters showed evidence of gastric distress. Although gastrointestinal injury is not routinely reported in association with exposure to chromate, animal data are supportive. In experiments conducted by Nettesheim et al. (1971) in mice, not only did exposure to calcium chromate dust generate additional subepithelial connective tissue and flattened epithelium in the large bronchi, but also atrophy of the spleen and liver. Ulceration of the stomach and intestinal mucosae also occurred. Furthermore, Mosinger and Fiorentini (1954) also observed acute gastroenteritis, as well as various lesions of the liver and kidneys, following the administration of potassium dichromate (2 mg Cr $kg^{-1}$) to various laboratory animals by a variety of routes.

Animal experiments illustrate that, generally speaking, hexavalent chromium compounds are more toxic than trivalent derivatives (NAS, 1974 and Tandon, 1982 provide extensive summaries of organ toxicities). Renal damage is most frequent, although necrotic changes in the liver have been reported. Isolated case reports of acute chromium poisonings confirm that both the liver and kidney are also susceptible to injury in man. A brief review of the human experience is provided below.

There are a number of reports in the literature of renal failure, liver failure and death of humans following ingestion of high doses of potassium dichromate. In 1935, for example, Goldman and Karotkin published a case of a 25-year-old woman, who, following ingestion of potassium dichromate, presented with gastrointestinal symptoms, renal and hepatic failure. The authors stated that this case was typical of the 69 cases previously reported. The evidence that acute renal failure follows ingestion of potassium dichromate is further exemplified by the clinical course of a young patient (Sharma et al., 1978). Oliguria lasted 12d. A percutaneous renal biopsy on the 18th day showed a loss of tubular epithelial lining, interstitial oedema and evidence of regeneration. The patient made a complete recovery.

In Section 3.3.6, a case study was described in which cutaneous absorption of trivalent chromium culminated in acute renal failure. A man was accidently immersed in hot acidic trivalent chromium sulphate solution but none was swallowed (Kelly et al., 1982). The clinical course was dominated by burns, intravascular haemolysis, and oliguric renal failure. Histological findings at necropsy revealed degeneration of liver, kidney and small intestine tissues, as well as acute lung congestion and intravascular coagulation. The authors concluded that the observed tissue chromium distribution (high levels in kidney and spleen, but lower in muscle and none in brain) was comparable to that reported for hexavalent chromium poisonings and correlated with the damage sustained. It seems reasonable to conclude that trivalent chromium is the renal toxic agent, even in chromate toxicosis.

Perhaps the results of Franchini et al. (1978) are of greater relevance (also described in Mutti et al., 1979). Urinalysis suggested that occupational exposure to chromium may have caused subtle renal dysfunction. Of 99 workers exposed to chromium compounds, none of the 39 stainless steel welders had evidence of abnormalities, but 8 of 36 workers using special chromium-alloy electrodes when welding had increased urinary levels of β-glucuronidase and 3 of these had actual proteinuria. Among 24 chromium platers, 9 had increased levels of β-glucuronidase, while 4 proteinuria. Similarly, Pascale et al. (1952) suggested that subtle hepatic dysfunction may occur in chromate-exposed workers, based on the appearance of acute hepatitis in a chromium electroplater and asymptomatic hepatic dysfunction in 4 coworkers examined. In these coworkers, hepatic tests and liver biopsies showed mild to moderate abnormalities; and all had nasal lesions typical of chromate exposure. At present, little can be said on the significance of these subtle kidney and liver changes, nor about the doses that may be expected to precipitate them.

On the basis of the evidence reviewed, it is clear that chromate, at least in high doses, is capable of inducing gastrointestinal damage, as well as kidney and liver dysfunctions. The identification of hepatic impairment and mild renal damage in workers exposed to chromate suggest the existence of a potential health hazard that warrants further in-depth evaluation and study.

## 6.6 Embryotoxicity, Teratogenicity and Other Deleterious Effects of Chromium on Reproduction

### 6.6.1 Synopsis

There is no human evidence at present that any form of chromium is associated with birth defects. However, the animal and other laboratory evidence reviewed in this section suggest that a cautious approach to the potential adverse effects of chromium compounds on reproduction is both judicious and warranted.

It was demonstrated in Chapter 4 that chromium is mutagenic in bacteria, yeast and cultured mammalian cells. Cytogenic effects in man are also observed. Imreh and Radulescu (1982), for example, performed cytogenic analyses on 18 workers from a potassium dichromate producing plant, and suitable controls. They found a significant increase in frequency of chromosome and chromatid aberrations in peripheral lymphocytes of workers with 20 years of exposure. In cultures of human lymphocytes, similar clastogenic effects were induced at concentrations of $K_2Cr_2O_7$ between 0.03 and 3.0 $\mu g\ ml^{-1}$. While sister-chromatid exchanges (SCEs) were produced in acute exposure *in vitro*, no significant increase was detected *in vivo* in the men with chronic occupational exposure. Sarto *et al*., (1982, 1983) have also detected chromosomal aberrations and SCEs in lymphocytes of workers exposed to chromic acid. Cr(VI) compounds are therefore genetically active. No studies of abnormal fetal outcome have been undertaken in occupational settings. Consequently, the studies on fetal outcome in animals experimentally exposed to chromium are therefore of considerable interest and these are now summarized.

### 6.6.2 Animal Studies

A number of animal and related studies have demonstrated that chromium(VI) compounds can be embryocidal and teratogenic (e.g., Matsumoto et al., 1976; Gale, 1978, 1982; Gale and Bunch, 1979; Gilani and Marano, 1979; Iijima et al., 1979). It has been shown, for example, that the frequency of a variety of malformations in the chick embryo at Day 8 increases with the dose of chromium(VI) oxide injected into the egg air sac at doses of 1 to 25 µg Cr on Days 0, 1, 2 or 3 of incubation (Gilani and Marano, 1979). Iijima and colleagues (1979), in a preliminary report on experiments with mice, found that chromium(VI) oxide injected subcutaneously at doses of 5 - 10 mg Cr $kg^{-1}$ on Day 8 of gestation had by Day 18 induced both embryocidal and teratogenic effects. Fetal growth was also impaired. Matsumoto et al. (1976) showed a positive dose-response relationship between amounts of chromium(III) chloride injected intraperitoneally on Day 8 of gestation in pregnant mice (10 - 24 mg Cr $kg^{-1}$) and the incidence in live fetuses on Day 18 of both external and skeletal abnormalities. Inhibition of fetal growth was also demonstrated. In a novel approach, Knudsen (1980) used a newly developed mammalian *in vivo* test for measuring genetic effects of chromates in the offspring of treated females. This test is dependent on transplacental transfer of the test compound, and assesses genetic alterations expressed as changes in fur pigmentation in the offspring. He found that intraperitoneal injection on Day 8 - 10 of gestation of welding fume particles (total of 100 mg $kg^{-1}$) containing 3.6% hexavalent chromium, as well as administering potassium chromate (total of 2.7 mg Cr $kg^{-1}$) produced significant positive results.

A number of studies of the embryotoxicity and teratogenicity of chromium(VI) oxide in hamsters illustrates the complexities inherent in animal work. Firstly, Gale (1978) has demonstrated a narrow dose-response range to chromium(VI) oxide administered intravenously to golden hamsters on Day 8 of gestation: a 2.5 mg Cr $kg^{-1}$ dose exerted minimal effects, while doses of 3.8 and 5 mg Cr $kg^{-1}$ were both embryolethal and teratogenic (cleft palate was the major malformation) and 7.5 mg Cr $kg^{-1}$ killed the pregnant animals. Secondly, the time of administration of the chromic acid was also a critical determinant of the embryotoxic and teratogenic responses (Gale and Bunch, 1979). And finally, Gale (1982) has also demonstrated that different strains of hamsters did not exhibit the same degree of susceptibility to chromium(VI) oxide-induced embryotoxicity and teratogenicity. Three strains were susceptible, while three others were resistant to these toxic events. It is obvious from the above experiments that extreme caution is necessary in attempting to extrapolate quantitatively animal results to humans.

Various investigators (e.g., Newbold _et_ _al_., 1979; Levis and Majone, 1979, and many others -- see Sections 3.3.5 and 4.2) have shown that survival and multiplication of cultured cells are affected in a dose-dependent manner by hexavalent salts, while it was considerably more difficult to demonstrate such effects with trivalent chromium. Jacquet and Draye (1982) confirmed that this is also the case in cultured mouse embryos by measuring the inhibition of blastocyst formation and hatching. The variety of deleterious effects induced by both hexavalent and trivalent chromium on fertilization and development in sea urchins has recently been studied by Pagano _et_ _al_. (1983). Sodium chromate was spermotoxic, suppressed mitosis and impeded differentiation (even when treatment was of sperm or eggs before fertilization). Aside from causing acute spermotoxicity, it is of interest that trivalent chromium severely reduced sea urchin embryo hatchability despite its lack of demonstrable effect on mitotic activity and differentiation.

In general, and as pointed out by IARC (1980) with respect to laboratory results such as those reviewed here, it is uncertain whether all the effects described represent direct insults on the embryo or fetus, or are a result of maternal toxicity. Matsumoto *et al*. (1976) point out that growth inhibition of specific chemical agents may reflect either (singly or in combination): (1) an alteration of some factor in the maternal system which secondarily affects fetal growth; (2) blocking of the placental transfer of some essential material necessary for normal fetal growth; or (3) a direct effect upon specific embryonic or fetal tissue. The identification and quantification of chromium in the fetus would help to resolve this matter. Unfortunately, placental transfer is often not determined (see below). Nevertheless, it seems that various types of chromium compounds have been linked with negative reproductive outcomes.

### 6.6.3 Placental Transport

It is recognized that chromium plays an important role in the function and growth of the body (as discussed in Section 3.3.7; Guthrie, 1982). It is also known that if adequate amounts of chromium are not taken by the mother during pregnancy, deficiency may result, which worsens with increasing parity (e.g., Saner, 1981; Wallach and Verch, 1984). Apparently chromium levels tend to be high in the newborn, then decline somewhat during the next decade or so (Schroeder *et al*., 1962). These authors have also reported measurable concentrations of chromium in some, but not all, fetuses of laboratory animals immediately after birth, suggesting placental transport during pregnancy. However, as indicated in Section 3.5.2.5, analytical problems might invalidate these observations. Placental transfer of chromium in either oxidation state was not observed in a number of early animal studies (e.g., Visek _et_ _al_., 1953; Mertz _et_ _al_., 1969). Mertz _et_ _al_. (1969) explained these seemingly contradictory observations by showing that placental transport depends on the specific form in which chromium is administered. For example, intragastric administration on Days 15 - 18 of gestation of $^{51}$Cr(III)-labelled brewers' yeast to pregnant rats led to significant labelling (20 ± 5%) of the newborn, compared to ~1% for chromium(III) chloride administered similarly, but daily, during gestation (also see Section 3.3.7). By contrast, administration of chromium(III) acetate in the drinking water during gestation and intravenous injection of chromium(III) chloride at mating did not result in appreciable transfer into the young. On the basis of these studies, IARC (1980) has concluded that inorganic chromium crosses the placenta only to a small extent, when administered to laboratory animals (regardless of oxidation state). The work of Matsumoto _et_ _al_., (1976) with chromium(III) chloride supports this conclusion (administered subcutaneously to mice during the first 16 days of gestation), since little additional chromium over controls was detected in the fetal bodies on Day 18.

Two recent studies have clarified the controversy concerning placental transport of chromium compounds. Polansky and Anderson (1980) have shown that radiolabelled chromium, administered to rats as chromium(III) chloride or sodium chromate, was transported across the placenta to the unborn pups, independently of the mode of administration (whether intravenously, subcutaneously, or intraperitoneally). Placental transport was time dependent and peaked when the dose was administered shortly before birth. Administration of the labelled chromium more than 10 days prior to birth resulted in insignificant transfer. Similarly, Wallach and Verch (1984) found that the mean $^{51}Cr$ content per placentofetal unit in rats was 0.89 ± 0.03% of the dose, injected intravenously on Day 17 of gestation and determined on Day 20. The results of these studies, together with those of Mertz *et al*. (1969), clearly indicate that time of administration is a major determinant in placental transport experiments. It may therefore be concluded that placental transport of chromium does occur in rats and is independent of the oxidation state in which it is administered. This conclusion is consistent with the evidence that chromium(III) and chromium(VI) compounds are embryotoxic and teratogenic in animals.

### 6.6.4 Concluding Remarks

In conclusion, there is some animal evidence that both chromium(III) and chromium(VI) compounds can be spermicidal, embryocidal or teratogenic. There seems to be a wide range of susceptibilities in this regard amongst different species. Placental transport in rats of chromium administered as chromium(III) or chromium(VI) has been demonstrated, but is strongly dependent on the time during gestation when the dose is given. The animal and related data reviewed suggest that judicious concern about the potential adverse effects of chromium compounds on human reproductive processes is warranted.

## 6.7 Summary

1. Chromate dust and chromic acid mist are responsible for the high prevalence of skin and nasal septum ulcerations among occupationally exposed individuals. Even very low atmospheric levels of water-soluble chromium(VI) compounds are associated with such skin and intranasal lesions (e.g. $\leq$ 0.015 mg Cr $m^{-3}$).
2. Other than acute chemical pneumonitis, which accompanies heavy exposure to chromic acid, little evidence exists to confirm the existence of non-malignant, chronic pulmonary disease from long-term exposure to chromium compounds.
3. Although gastrointestinal, hepatic and renal dysfunctions occur on acute chromate poisoning, their prevalence among workers is uncertain (even in mild form).
4. There is no human evidence that chromium compounds are associated with birth defects, or other reproductive deficits. Animal experiments have

demonstrated both chromium(III) and chromium(VI) compounds can be spermicidal, embryocidal and teratogenic. Placental transport of chromium has been demonstrated in rats for both oxidation states.

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**Chromium**

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as transient intermediates: 3/14, 28

stability: 3/3, 14

**Chromium(III) oxide, $Cr_2O_3$**

phagocytosis of: 4/40

**Chromium(VI) oxide, $CrO_3$**

as irritant: 3/6

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of Cr(III) and Cr(VI) compounds: 4/4-9, 46-54, 74, 97-9

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in analysis: 3/46-7, 93

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Dichromate ion

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H

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M

Metal chromates

water solubility: 2/2; 3/5, 92

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of Cr(III) and Cr(VI) compounds: 4/4-9,46-54,74,97-9

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determination: 3/48-50

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correlation with urinary Cr: 3/65-7

  determination: 3/48-50

  levels: 3/44, 51, 65-7, 79

Solubility

  of metal chromates: 2/2; 3/5, 92

Spermotoxicity: 1/3; 6/17-23

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Threshold concentrations

  for ulcerations: 2/13; 6/6-8

U

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